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VACCINE EFFECTIVENESS AGAINST HPV INFECTIONS

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Vaccine Effectiveness against HPV Infections

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

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To Michael Kann: my beloved husband, my friend and my meaning of life

ABSTRACT

Infections with human papilloma viruses (HPV) are the most prevalent sexually transmitted diseases. The vast majority of the sexually active population acquires them during lifetime but almost all HPV infections become resolved within a year. A small fraction of infections persists and can lead to cervical lesions, which may progress to cancer and their appearance is controlled by long-established cervical screening programs. Since licensure of the first HPV vaccines almost 15 years ago, more than one hundred countries introduced HPV vaccination into their national immunization strategies in order to prevent cervical cancer development. Despite permanent evaluation of immunological, virological and clinical endpoints of vaccine effectiveness, the antibody dynamics and protection are not fully understood, which is particularly true after natural infection. We thus studied humoral immune responses after natural infection and vaccination. In addition, we investigated reasons for rare events of cervical lesion development after immunization.

In studies of **serological responses to HPV infections** (papers I and II) we demonstrated that the characteristics of type-specific anti-HPV antibodies to the most common oncogenic types (HPV 16 and 18) apply also to the other high-risk HPVs. Serum antibodies were generally stable over time and were strongly associated with cervical HPV DNA positivity. Seropositivity to multiple anogenital HPV types was also associated with presence of HPV DNA and we observed an association with an abnormal cytology and with the presence of non-genital HPVs. The latter finding suggests that a definite subset of women is more likely to seroconvert to multiple HPV types, which could be true also in induction of antibody responses to HPV vaccines.

Our serology assay was validated using HPV DNA from serially collected cervical samples as a standard. This was shown to be a valuable approach for evaluation of assay performance since repeated sampling for transient exposure may improve sensitivity without impairing specificity. In consequence, our multiplexed HPV serology assay was expected to be useful in studies of immune responses to HPV infection and vaccines.

Our research on **immunogenicity of the HPV vaccines** was a head-to-head comparison of the anti-HPV antibody responses between the quadrivalent Gardasil and bivalent Cervarix (papers III and IV). We evaluated the sustainability of vaccine-targeted and cross-reactive serological responses up to 12 years post-vaccination. We found that the antibody levels induced by Cervarix were stable and virtually all women had anti-HPV antibody titers above those induced by HPV infection. In contrast, most of the recipients of quadrivalent Gardasil had detectable antibody levels but 8 % (HPV-16) and 18 % (HPV-18) showed a titer-decline to below the natural infection antibody level. Comparison of the antibody responses exhibited that the anti-HPV-16 antibody levels were 5.1-fold higher and anti-HPV-18 were 18.4-fold higher in the recipients of bivalent Cervarix ($p < 0.0001$). Further, the seropositivity to most of the non-vaccine HPV types was more prevalent among the women vaccinated with Cervarix than with the Gardasil. Nonetheless, anti-HPV antibody levels and avidity were comparable for almost all HPV types. For both vaccines, we found that the seroprevalence to cross-reactive types (HPV-31, -33, -35, -45, -52, -58, -59, -68 and -73) increased with the anti-HPV-16 levels. In the recipients of the bivalent HPV vaccine, antibody levels of the non-vaccine types HPV-31, -35, -51, -52, -56 and -58 increased with the anti-HPV-16 levels, which was not observed in women vaccinated with the Gardasil. Furthermore, our investigations showed that anti-HPV antibody levels shortly after vaccination predicted antibody levels and antibody avidity a decade later.

Lastly, we investigated **whether the development of cervical lesions after HPV vaccination is associated with the vaccine-targeted HPV types** (paper V). In this research project, we studied a cohort of women immunized with HPV vaccine before 17 years of age. We observed that not a single woman was positive for the low-risk vaccine types HPV-6 and -11. We found that cervical lesions were usually not associated with the vaccine-targeted HPV types of the highest oncogenic risk. Instead, non-vaccine types having a lower potential for inducing progression to cervical cancer were more likely to be present. Evidently, the latter finding has significant implications for the future design of cervical screening strategies that would target highly vaccinated cohorts of women and for design of surveillance and monitoring strategies. Another consequence of these findings is the information to physicians and vaccinated women that cervical dysplasia may occur despite HPV vaccination but likely caused by HPV types that rarely progress to cancer.

In summary, the findings presented in this thesis contribute to the understanding of the dynamics and the durability of naturally acquired and vaccine-induced antibody responses to HPV. The results give reasons for the cervical lesion-development among young HPV-vaccinated women. We conclude that the future definition of a clinically relevant anti-HPV antibody protective threshold, as well as the evaluation of determinants for higher and broader immunological responses to HPV vaccines are necessary to optimize HPV vaccine implementation and cervical screening strategies.

LIST OF SCIENTIFIC PAPERS

I. Artemchuk H, Triglav T, Oštrbenk A, Poljak M, Dillner J, Faust H. Seroprevalence of Antibodies to 11 Human Papillomavirus (HPV) Types Mark Cumulative HPV Exposure. J Infect Dis. 2018; 218(3):398–405.

II. Faust H, Artemchuk H, Oštrbenk A, Triglav T, Poljak M, Dillner J. Seropositivity to Multiple Anogenital Human Papillomavirus (HPV) Types Is Associated With Current Anogenital HPV Infection, Abnormal Cytology, and Seropositivity for Nongenital HPVs. J Infect Dis. 2019; 219(3):489–496.

III. Artemchuk H, Eriksson T, Poljak M, et al. Long-term Antibody Response to Human Papillomavirus Vaccines: Up to 12 Years of Follow-up in the Finnish Maternity Cohort. J Infect Dis. 2019; 219(4):582–589.

IV. Kann H, Lehtinen M, Eriksson T, Surcel HM, Dillner J, Faust H. Sustained Cross-Reactive Antibody Responses After Human Papillomavirus Vaccinations: Up To 12 Years Follow-Up In The Finnish Maternity Cohort. Accepted to the Journal of Infectious Diseases.

V. Kann H, Hortlund M, Eklund C, Dillner J, Faust H. Human Papillomavirus Types In Cervical Dysplasia Among Young HPV-Vaccinated Women: Population-Based Nested Case–Control Study. International Journal of Cancer. 2020; 146(9):2539–2546.

SCIENTIFIC PAPERS WHICH ARE NOT INCLUDED IN THESIS COMPILATION

I. Triglav T, Artemchuk H, Oštrbenk A, et al. Effect of naturally acquired type-specific serum antibodies against human papillomavirus type 16 infection. J Clin Virol. 2017; 90:64–69.

II. Gray P, Kann H, Pimenoff VN, et al. Long-term follow-up of human papillomavirus type replacement among young pregnant Finnish females before and after a community-randomised HPV vaccination trial with moderate coverage. Int. J. Cancer. 2020; 1– 12. https://doi.org/10.1002/ijc.33169

III. Gray P, Kann H, Pimenoff VN, et al. Gender-Neutral Vaccination Is The Key To HPV16 Herd Effect When Vaccination Coverage Is Limited: A Community-Randomised Trial Of Vaccination Strategy. Submitted.

IV. Mariz FC, Gray P, Bender N, et al. Sustainability of neutralizing antibodies induced by bi- or quadrivalent HPV vaccines correlates with efficacy. Submitted.

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LIST OF ABBREVIATIONS

Abbreviation	Explanation
AI	avidity index
AU	arbitrary assigned unit
BVR	bivalent (Cervarix HPV vaccine) vaccine recipients
CI	confidence interval
CIN	cervical intraepithelial neoplasia
cLIA	competitive Luminex immunoassay
CV	coefficient of variation
ELISA	enzyme-linked immunosorbent assay
EMA	European Medicines Agency
FMC	Finnish Maternity Cohort
FUTURE II	quadrivalent HPV vaccine clinical trial: Females United to Unilaterally Reduce Endo/Ectocervical Disease
HPV	human papillomavirus
HR HPV	high-risk human papillomavirus
HSIL	high grade squamous intraepithelial lesions
IARC	International Agency for Research on Cancer
ICC	invasive cervical cancer
ICTV	International Committee on Taxonomy of Viruses
ID	identification number
IgA, IgM, IgG	immunoglobulins of classes A, M, G
IRF	interferon regulatory factor
IS	international standard

IU	international unit
JCPyV	JC polyomavirus
LR	low-risk human papillomavirus
LSIL	low grade squamous intraepithelial lesions
MCPyV	Merkel Cell Polyomavirus
MFI	median fluorescence intensity
MGP (GP5+/6+)	modified general primers for HPV DNA amplification
NKCx	National Cervical Screening Registry, Sweden
NVR	National Vaccination Register, Sweden
OR	odds ratio
ORF	open reading frame
PAF	population attributable fraction
PATRICIA	bivalent HPV vaccine clinical trial: PApilloma TRIal against Cancer In young Adults
PBNA	pseudovirion-based neutralization assay
PCR	polimerase chain reaction
PDR	Prescribed Drug Register, Sweden
PHA	Public Health Agency, Sweden
PIN	personal indentifying number
QVR	quadrivalent (qGardasil HPV vaccine) vaccine recipients
RVI	Register of Vaccinated Individuals, Finland
RWE	real-world evidence
SNOMED	Systematized Nomenclature of Medicine
SOP	standard operating procedues

THL	Finnish Institute for Health and Welfare
TLR	toll-like receptor
VE	vaccine efficacy
VLP	virus-like particle

1 BACKGROUND

1.1 HPV AS A CAUSATIVE AGENT OF HUMAN CANCERS

Human papillomaviruses (HPVs) are small non-enveloped DNA viruses, which infect human epithelial cells. In some cases they escape from the host immune surveillance, persist in basal keratinocytes over long periods of time and, rarely, cause malignant diseases at different anatomical sites, most notably in cervix [1]. In 1983, the first laboratory evidence suggested a causative link between HPV16 infection and cancer stating that "the regular presence of HPV DNA in genital cancer biopsy samples does not *per se* prove an etiological involvement of these virus infections, although the apparent cancer specificity of HPV16 is suggestive of such a role". This observation shifted focus from the Herpes Simplex Virus, which was the main suspect causing genital cancers [2] to the rapidly growing family of human papillomaviruses. Accumulated numerous biological and epidemiological evidences fulfilled Hill's criteria for establishing a causal relationship between HPV infection and a subset of human genital and oropharyngeal cancers [3].

The International Agency for Research on Cancer classified genital HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59 as carcinogenic, and HPV 68 as probably carcinogenic to humans [3]. These HPV types are also frequently referred to as high-risk HPV (HR-HPV). Similarly, HPV types which do not associate with human cancers are called low-risk (LR-HPV), comprising HPV 6, 11, and others. HPV 6 and 11 usually cause only benign epithelial diseases, such as anogenital warts and recurrent respiratory papillomatosis [4].

Invasive cervical cancers rank the fourth most common cancers in women worldwide leading to an estimated half a million new cases per year [5,6]. Recent estimates demonstrate that practically 100 % of cervical cancer cases, 88 % of anal, 78 % of vaginal, 50 % of penile, 25 % of vulvar and 31 % of oropharyngeal carcinomas are attributable to infection with HPV [5]. The total burden of HPV-associated cancers is translated into 570,000 - 620,000 new cases per year in women and 60,000 - 70,000 cases in men [7,8]. Exemplified for female population, the geographical distribution of proportions of cancer cases attributable to infections with HPVs in 2018 are shown in **Fig. 1** [8,9]. Age-standardized incidence rates of these HPV-associated cancers are inversely associated with the national income levels of the countries, highlighting that cervical cancer screening and HPV vaccination programs in low-income countries have a huge preventive potential [8].

1.1.1 Human papillomaviruses in all their phylogenetic beauty

Viruses of *papillomaviridae* family exhibit a strong host- and cell-type tropism, infecting basal epithelial cells of a wide range of vertebrates, including humans [10]. By the degree of nucleotide sequence homology of the most conserved L1 gene, HPVs are classified into genera, species and types [11]. HPVs from different genera share 45 – 60 % similarity of L1 sequence, within a genus (species) – between 60 and 70 % [11]. A novel HPV type has less than 90 % similarity to any other HPV type [12]. Beyond type, homology between 90 % and 98 % defines a subtype, and - more than 98 % - a variant [11,12].

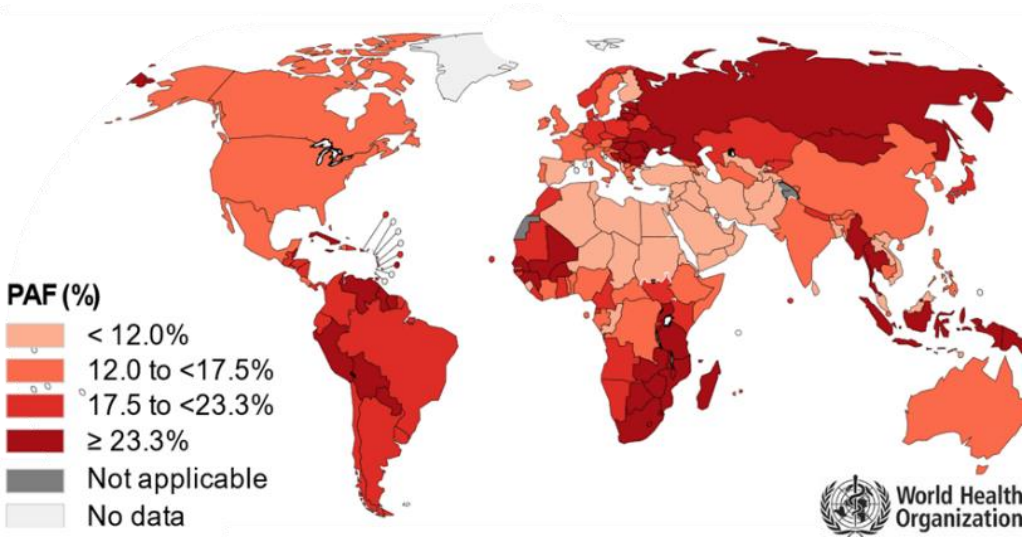

Figure 1. HPV infection population attributable fraction (PAF, %) for all cancer cases among females, 2018 [8]. The boundaries and names shown and the designations used on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted and dashed lines on maps represent approximate border lines for which there may not yet be full agreement. Data visualization (adapted): IARC / GCO, World Health Organization [9].

Established by the International Committee on Taxonomy of Viruses (ICTV), the five known HPV genera are marked by Greek letters (Alpha-, Beta-, Gamma-, Mu- and Nu papillomavirus) [13]. Except of Alpha papillomaviruses, all of genera are mainly associated with cutaneous infections [1]. On the lower order of classification, species naming is formed by the name of genera which is followed by an integer, e.g. Alpha-9 [11]. HPV types are assigned their names (e.g., HPV 16) by the International HPV Reference Center [12,14] and the unique numbers are based on the numerical order of their discovery. To date, at least 226 types of HPV are identified, cloned and stored in the human papillomavirus reference center [12].

1.1.2 Virus structure and lifecycle

HPV is a non-enveloped icosahedral virus of about 55 nm in diameter [10]. The life cycle of HPV is confined to epithelial cells, with replication occurring only in fully differentiating squamous keratinocytes [15]. The viral genome consists of about 8 kbps of double-stranded circular DNA comprising coding and non-coding regions (**Fig. 2**) [1]. The latter consists of the upstream regulatory region (URR), which is a sequence of regulatory elements controlling early gene transcription and replication [15,16]. The coding regions are separated in two classes of viral genes encoding early (E1, E2, E4, E5, E6, E7) and late (L1 and L2) proteins.

Early events of the viral life cycle depend on the accessibility of the basement epithelial membrane and of basal keratinocytes, that may become exposed upon micro-wounding or abrasion [17]. The viral capsid requires contact with heparin sulphate proteoglycans [18], followed by furin cleavage of L2 capsid protein resulting in structural changes of the capsid [19]. Interactions with secondary cellular receptors such as annexin A2 [20,21], growth factor receptors [18], CD151 (a member of the transmembrane 4 superfamily) [22,23] allow cell entry by endocytosis [24], and subsequent release into the cytoplasm. Entry of the viral DNA into the nucleus – which is a prerequisite for replication – occurs during nuclear envelope break-down upon mitosis [25].

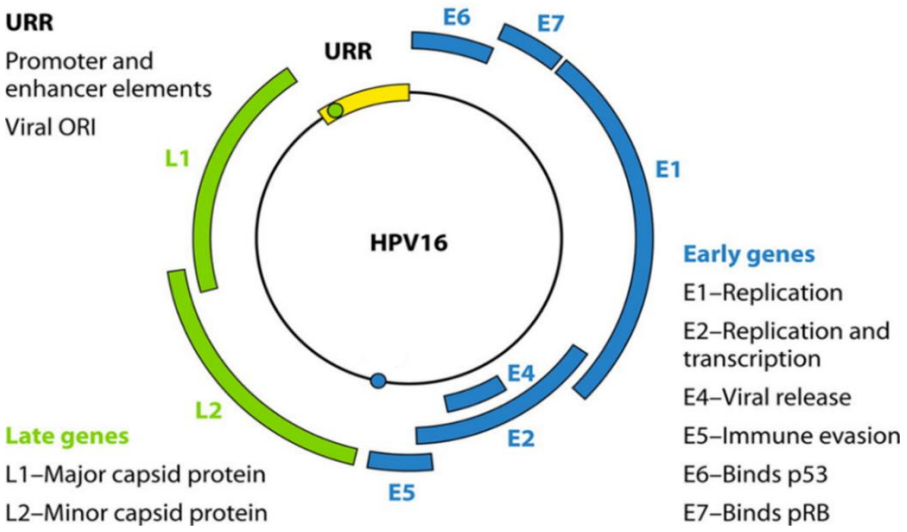

Figure 2. Genomic organization of a typical mucosal high-risk HPV and main functions of the proteins encoded by viral early and late genes [15]. HPV, human papillomavirus; URR, upstream regulatory region; viral ORI, viral origin of replication; E1-7, genes encoding early viral proteins; L1-2, genes encoding late viral proteins. Early genes are transcribed in the lower layers of epithelia and late genes – in the upper. Adapted from M. Stanley, Epithelial Cell Responses to Infection with Human Papillomavirus, Clin Microbiol Rev Apr 2012, 25 (2) 215-222, reproduced with permission from the American Society for Microbiology, RightsLink Copyright Clearance Center.

Once HPV successfully infected the keratinocytes, its life cycle becomes aligned with the stages of epithelial differentiation: genome maintenance (basal cells), viral protein production (suprabasal layers of epithelia), followed by virion assembly and release (upper layers of skins/mucosa) (**Fig. 3**) [26]. During maintenance, early proteins E1 and E2 control viral genome replication and episome maintenance. In the following stages of the lifecycle, proteins E4 and E5 support virus release by disrupting cytokeratin structure and contribute to genome amplification, respectively [27]. Products of E5 gene expression also interact with epidermal growth factor receptors, mediate immune evasion and induce koilocyte formation [1,27,28]. Major (L1) and minor (L2) capsid proteins package the genome and, after intracellular maturation, newly created virions are ready for shedding [1,16].

However, in HR HPV types, E6 and E7 may also be expressed in early stages, inducing basal cell proliferation [1,16]. Deregulation of E6 and E7 expression leads to binding and degradation of tumour suppressor proteins p53 and pRb, respectively. Impaired functionality of p53 in turn results in inhibition of apoptosis; absence of pRb induces genome instability and cell cycle entry.

1.1.3 Persistence of HPV infection and disease progression

Worldwide, HPV infections are the most common among sexually transmitted infections and a large proportion of the sexually active population acquires such HPV infection(s) during lifetime [29]. Epidemiological evidence suggests that within three years from sexual debut up to 44 % of adolescent girls become HPV DNA positive to at least one of the eight HPV types (HPV 6, 11, 16, 18, 31, 33, 52, 58) [30]. However, most women clear HPV infections within approximately one year [30,31].

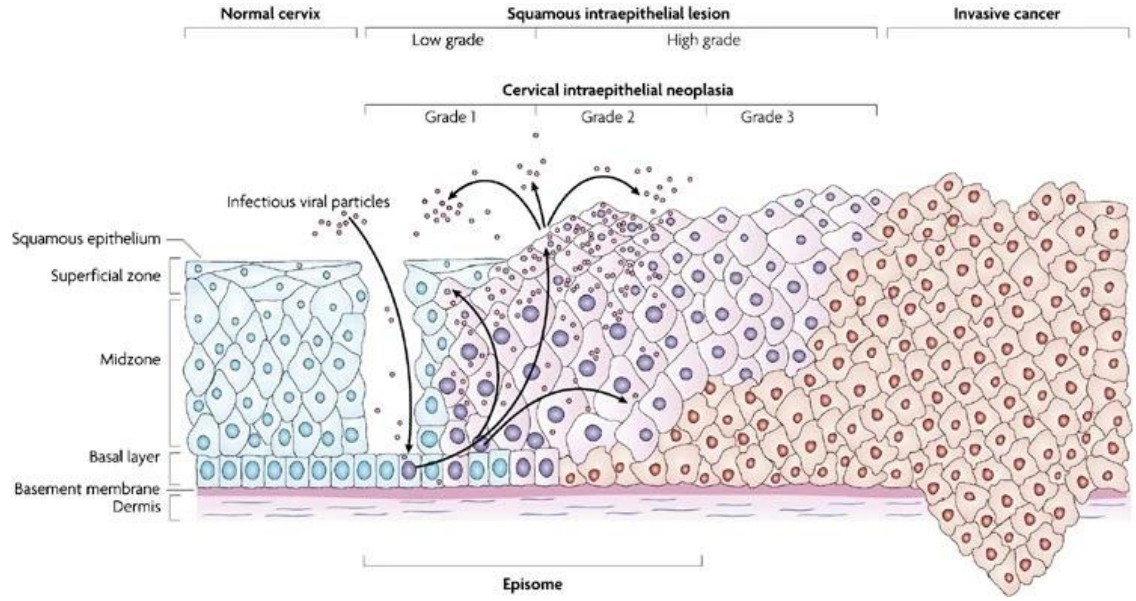

Figure 3. Scheme of the course of progression of HPV infection to cervical cancer [32]. Basal epithelial cells rest on the basement membrane above dermis. Human papillomavirus (HPV) access these cells through micro-wounds. During the early stages of infection, the HPV genes E1, E2, E5, E6 and E7 are expressed, which maintains viral DNA replication from the episomes (purple nuclei). As epithelial cells differentiate, in the upper layers the viral genome is further replicated, and genes E4, L1 and L2 are expressed. Products of expression of late genes (L1 and L2) encapsidate the viral genomes to form progeny virions in the nucleus, which leave the cell upon its death and can then initiate a new infection. Low-grade intraepithelial lesions support viral replication. Persistent infections with high-risk HPV types may progress to high-grade cervical intraepithelial neoplasia, microinvasive and invasive cancer and is associated with the integration of the HPV genome into the host chromosomes (red nuclei), loss or disruption of E2, upregulation of E6 and E7 oncogene expression. Adapted from Woodman, C., Collins, S. & Young, L. The natural history of cervical HPV infection: unresolved issues. Nat Rev Cancer 7, 11–22 (2007), reproduced with permission from the Springer Nature, RightsLink Copyright Clearance Center.

Time of HPV infection clearance varies between geographical regions and may depend on a number of biological and behavioral determinants, such as the type of HPV, the viral load, previous HPV infections, co-infection with high-risk HPV type, HIV status, site of infection, number of sex partners, etc. [31,33–37]. The HPV type-dependent time to clearance was demonstrated in an early study of the natural history of cervical HPV infections: the estimated time of HPV clearance in HPV DNA-positive girls after adolescence was c. 10 months for HPV-16 and almost 8 months for HPV-18 [30]. According to a meta-analysis, these intervals are longer, reaching on average a year for HPV-16 and almost ten months for HPV-18, with no distinguishable trend of HPV persistence by age [31].

Persistent infection with HR HPV types is a main factor determining progression to cervical cancer, and several co-factors may contribute to it. As reviewed by the IARC working group on human carcinogens, the co-factors for infection persistence and progression to cervical lesions include cigarette smoking, number of pregnancies, immunosuppression and exposure to other infectious agents [3]. Evidence for the other co-factors, such as the use of hormonal contraceptives, exposure to certain nutrients (e.g., folate), as well as genetic determinants, are not consistent across the studies [3].

Once HPV infection cannot be cleared and persists for a year or more, the risks for malignant transformation increase [30,38,39]. Cervical lesion development towards cancer undergoes three consecutive stages of malignant disease precursors called cervical intraepithelial neoplasia (CIN) of grades 1-3 (**Fig. 3**) [32]. When the neoplasia grade increases, the probability for persistence and progression towards the next grade and towards cervical cancer becomes also higher [39]. Inversely, the probability of lesion regression decreases over the course of neoplastic development, ranging between 40 % for the CIN 2 and 33 % for the CIN 3 lesions [39,40]. The course of disease progression is reflected by the classification of cervical epithelial cell abnormalities (Bethesda classification) used in clinical practice:

- low grade squamous intraepitelial lesions (LSIL), which encompass cytological changes that correspond to HPV infection/mild dysplasia/CIN1;

- high grade squamous intraepithelial lesions (HSIL), which encompass moderate and severe dysplasia, CIS (carcinoma in situ), CIN 2 and CIN 3, and, if invasion is suspected, may have features of invasion;

- squamous cell carcinoma [41].

A six-months persistent infection with HR HPV types is associated with highly elevated risks for development of precancerous lesions and cervical cancer [35,36,42]. Baseline presence of HPV-16/-18 DNA confers 8.5-fold and 18.6-fold increased risks of cervical cancer *in situ* and invasive squamous cell carcinoma, respectively, compared to HPV DNA negative women [42]. This observation is consistent with the findings that HPV-16 and -18 are the most abundant papillomavirus types in cervical cancers, accounting for about 70-75 % of all invasive cervical cancer (ICC) cases [3]. In the remaining 25-30 % of cervical cancer biopsies, the descending prevalence of HPV types is: HPV-33,-45,-31,-58,-52,-35,-59,-51,-56,-39,-68 [3].

Overall, HPV-16 is one of the most persistent, as well as the most frequent type in cervical, anal, vulvar and vaginal lesions [31,35]. The proportion of HPV-16 DNA positivity in cervical sample of HPV-positive women increases with the augmentation of severity of cytological and histological findings, being around 20 % in normal cytology and atypical squamous cells of undetermined significance, 25 % in low-, 48 % in high-grade squamous intraepithelial lesions, and 63 % in the ICC biopsies [43]. A similar trend was observed for the frequency of HPV DNA integration into the host chromatin, which increases with cervical disease severity [1,32].

It appears, however, that the subset of cervical cancers it too heterogeneous for referring to as a single disease and the risk for cancer development is not only HPV type-dependent but is related to the origin/location of the initially infected cervical cells (basal cells of the ectocervix *versus* cuboidal cells at the squamo-columnar junction vs columnar cells of the endocervix) [44]. In particular, the origin of the infected basal cells has recently been suggested to be one of the major determinants for the speed of cervical lesion progression but more research is needed to elucidate underlying mechanisms [44].

1.2 IMMUNE RESPONSE TO HPV

The immune system controls HPV infection by both innate and adaptive answers of the immune system (**Fig. 4**) [45]. It responds to HPV infection by initiating the innate chain after detection of damage or by TLR-mediated sensing the pathogen-associated molecular patterns (TLR1, TLR3, TLR7, TLR8, TLR9, as well as TLR2) [15,46–48]. At this stage, immediate non-

specific effectors are activated, and type I INF-α and-β are secreted. Then, proinflammatory cytokines and chemokines support activation of local antigen presenting (APC) Langerhans' cells which bring the processed viral fragments to locoregional lymph nodes. Subsequent activation of CD8 cytotoxic T cells and antibody-secreting B-cells is mediated by T helper cells (**Fig. 4**). Cellular immunity clears HPV infection from the infected sites and humoral chain of adaptive immunity generates neutralizing mucosal antibodies which prevent HPV infection in future [45].

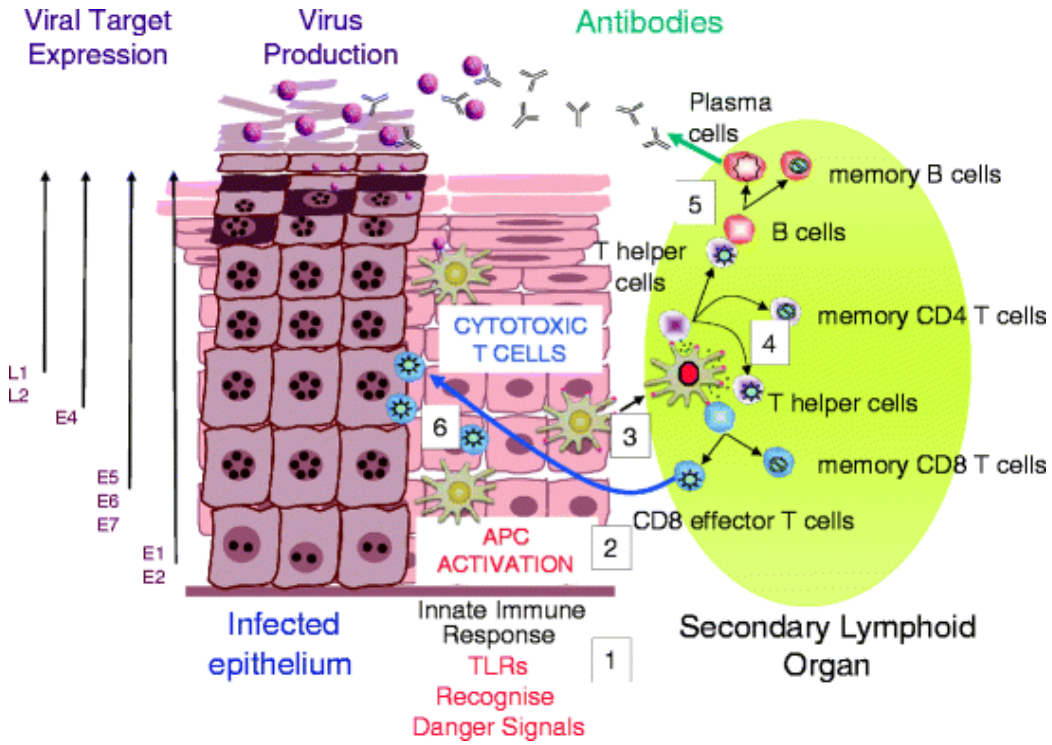

Figure 4. Scheme of main steps in natural immune control of HPV infection [17,45]. (1) Innate immune system recognizes damage of epithelium. (2) Immediate non-specific effectors are activated, and interferons are secreted. (3) Proinflammatory molecules promote activation of Langerhans' cells, processing of the antigen and migration to locoregional lymph nodes, which further (4) activates the adaptive chain of immunity. Specific CD4 T helper 1 type immunity supports development of effector and memory CD8 cytotoxic T cells against early viral proteins. (5) In addition, T helper cells also support activation of B cells that secrete neutralizing antibodies. Long lived plasma cells produce high levels of antibodies against HPV. These antibodies transudate into the mucosa and protect against re-infection (6). Reproduced from Stern P.L., Einstein M.H. (2012) The Immunobiology of Human Papillomavirus Associated Oncogenesis. In: Borruto F., De Ridder M. (eds) HPV and Cervical Cancer. Springer, New York, NY. https://doi.org/10.1007/978-1-4614-1988-4_3, with permission from the Springer Nature, RightsLink Copyright Clearance Center.

HPV developed passive and active ways for evading host's immune system [45,49]. Examples for passive immune escapes are: i) absence of viremia and thus no widespread infection, ii) no lytic replication step, and no inflammation. In addition, iii) rare codons result in low level viral protein expression, and iv) by the time virions are shedding on upper layer of the epithelium, they are not accessible for immune cells anymore [45,49]. Recently, another, novel humoral immune escape mechanism through seroconversion to L1 isoforms of different length, was described [50].

Active ways to escape the immune response are mainly carried through hampering IRF, IFN and NF-kB signaling through the early HPV proteins E2, E5, E6 and E7, as well as through impairing antigen presentation by absence of activation/recruitment/sustainment of antigen presenting cells (E5 and E7) [15,49,51].

1.2.1 Naturally acquired anti-HPV antibody responses

The ability to evade from the recognition by the immune system at least partially explain why about 40 % of HPV-16 infections in females do not result in seroconversion [52]. Among those who become seropositive, it may take up to 1.5 years to mount measurable antibody responses [52], which are usually stable over time [53–55]. Compared to transient HPV infections, persistent are more likely to induce seroconversion [56]. Evidence from the case-control study shows that anti-HPV antibodies to HR types 16 and 18 are associated with the higher odds for development of cervical cancer [57]. Potential protectivity by the naturally acquired immunity against the incident and persistent infections appears to be inconsistent across the HPV types and across the studies [58–61]. However, meta-analysis demonstrated significant protection against subsequent infection with HPV-16 (pooled RR, 0.65; 95 % CI 0.50–0.80) and HPV-18 (0.70; 0.43–0.98) in women [62].

HPV type-specific seroconversion is a slow process: subjects with an incident HPV-16 infection seroconvert to IgG within 8.3 months (maximum of 56.7 % positivity) and to IgA within 14 month (maximum of 37.0 % positivity) [63]. Acquisition of anti-HPV antibodies is associated with HPV DNA positivity (same-type or the phylogenetically related genotypes), with persistence of HPV infection and with higher viral load [63]. Seroepidemiological features of HPV-16/18-infections were extensively studied but less is known about the other HR HPV types [53,64–66]. Genotype-specific estimates of the association between seropositivity and HPV infections are scarce, inconsistent across the assays, and vary between the different HPV types even when the same method is used [67–71]. For instance, in multiplex serology assay based on glutathione S-transferase fusion protein, type-specific agreement of HPV DNA positivity and seropositivity was observed for HPV-33, -52 and 58 but not for HPV-16, -18, -31 and -45 [68].

1.2.2 Humoral immune responses to VLP-based HPV vaccines

Quadrivalent Gardasil (HPV-6, -11, -16, -18; Merck &Co, Kenilworth, NJ, USA/Sanofi Pasteur MSD, Lyon, France) and bivalent Cervarix (HPV-16, -18; GlaxoSmithKline Biologicals, Wavre, Belgium) vaccines were licensed almost 15 years ago. In a more recent nonavalent Gardasil, additional five HR types HPV-31, -33, -45, -52, -58, as well as higher amounts of adjuvant and antigens, are included.

All these vaccines are based on virus-like particles (VLPs), which use the property of spontaneous self-assembly of L1 proteins after their overexpression in mammalian- and in insect cells (Cervarix) or in yeast (Gardasil). Aside of the included HPV types, the abovementioned vaccines also differ in their adjuvants, which are aluminiumhydroxide and phosphorylipid AS04 in Cervarix and aluminiumhydroxy-diphosphosulfate in Gardasil.

Evidently, each of the three licensed HPV VLP-based vaccines is safe and immunogenic [72–77]. Comparison of the two Gardasil vaccines showed that antibody levels to HPV-6, -11, -16, and -18 in the nonavalent vaccine recipients are non-inferior to those of the quadrivalent vaccine recipients (QVR) up to three years after vaccination [77]. Vaccine efficacy (VE) was demonstrated against incident and persistent HPV infections, as well as genital warts (Gardasil vaccines only), cervical neoplasms and, preliminarily, invasive HPV-related cancers [77–90].

VLP-based HPV vaccines induce high levels of high-avidity antibodies with neutralizing properties [77,91–93], which transudate to the mucosal surfaces. By blocking different viral surface epitopes, these antibodies prevent HPV from interaction with the membrane receptors and from binding to the cells [94–96]. This mechanism of viral epitope blocking was demonstrated in *in vitro* neutralization assays [97–99], in the murine genital-challenge model using passive transfer of neutralizing antibodies[100], and among the HPV vaccine recipients by comparing systemic anti-HPV antibody levels with local in the cervicovaginal secretions [91,94,101,102]. Both in the BVR and in the QVR, systemic and mucosal antibody levels correlate strongly (R^2 for HPV-16 = 0.73 - 0.97; HPV-18 = 0.69 - 0.96). Notably, also for the non-vaccine HPV types, antibody concentrations in the cervicovaginal secretions correlate with the serum levels (R^2 for HPV-31 = 0.84; HPV-45 = 0.71) [91].

HPV vaccines induce long-lasting cellular and humoral responses: the presence of reactive memory B cells was demonstrated by an antigen challenge with the quadrivalent vaccine five years after the initial three-dose immunization [103]. It yielded a fast and strong humoral response: one week after the booster dose, antibody levels reached titers observed one month after the initial full-dose vaccination. One month post-challenge, anti-HPV antibody levels exceed those obtained after the three-dose immunization [103]. At least up to ten years from immunization, post-vaccination antibody responses to HPV remain above levels acquired after infection [92,104–110].

Cross-protective VE against phylogenetically related non-vaccine genotypes was demonstrated for the bivalent and the quadrivalent HPV vaccines, although it has been weaker than VE against HPV-16/-18 [78,84,85,111]. The evidence for cross-protection is more consistent among the bivalent vaccine recipients (BVR) than the QVR [81,83,85–90,112–114]. In line with this, evidence from the HPV vaccine immunogenicity studies demonstrates that Cervarix induce higher antibody levels than the quadrivalent Gardasil [91,101,110,115,116]. In the short-term vaccine comparison studies, neutralizing anti-HPV antibody levels of vaccine-targeted HPV-16 and -18, as well as non-targeted HPV-31, -33-, 35, -45, -52, -58, were higher in the BVR than in the QVR [91,117]. Scarce data from the long follow-up studies further reported higher seroprevalences to HPV-31 and -45 in the BVR than in the QVR [118]. Collectively, sustainable cross-reactive immune responses against HPV-31,-33,-45,-52,-58 were reported for the bivalent HPV vaccine [105,119], however among the QVR it was applicable mainly for HPV-31 [120,121]. The major drawbacks in cross-reactive immunogenicity studies of the HPV vaccines are their relatively short time of follow-up and a limited number of HPV types under evaluation [118,122,123].

HPV vaccine-induced serological responses which are typically 10 - 100 times higher than the antibody levels after HPV infection (**Fig. 5**) [124–127]. This was demonstrated for the single-double- and triple-dose immunization schedules [125,127] and the results from the randomized clinical vaccine trials [128–130] prompted WHO to update the recommendations of HPV vaccination schedules towards the two-dose regimen. This is currently adopted in many organized immunization programs, although vaccination of the immunocompromised follows a three-dose schedule [131]. In addition, since anti-HPV antibody responses in the group of older females (age ≥ 15 years) were demonstrated to be lower compared to the girls vaccinated at younger age [94,132], vaccine recipients of more than 15 years of age should also stay with three-dose regimen [131].

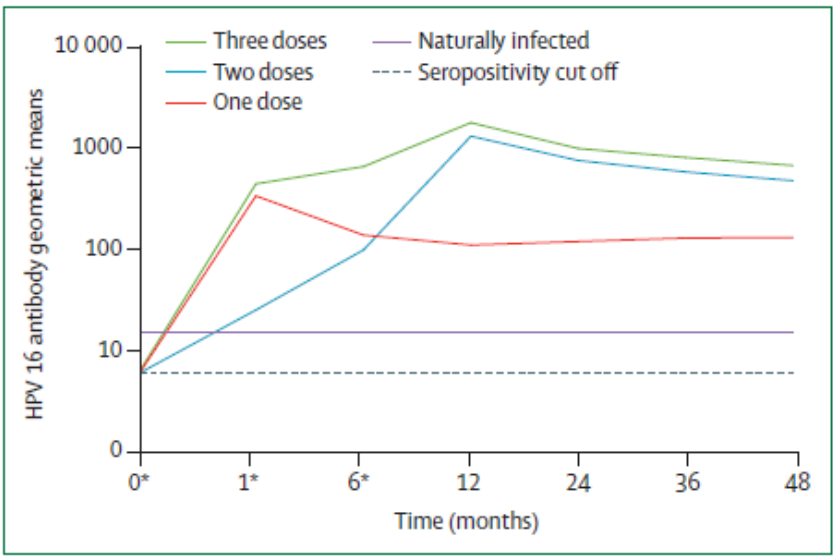

Figure 5. HPV-16 virus-like particle antibody geometric means induced by the bivalent vaccine in 18–25 years old women enrolled to the Costa Rica clinical trial [127,133]. Three-dose immunization was performed at months 0, 1, and 6; two-dose – at months 0 and 6; single-dose – at month 0. HPV, human papillomavirus. Timeline of vaccination is marked with asterisks [127,133]. Reproduced from Schiller JT, Müller M. Next generation prophylactic human papillomavirus vaccines. The Lancet Oncology. 2015; 16(5):e217–e225, with permission from the Elsevier, RightsLink Copyright Clearance Center.

A post-hoc analysis of the Costa Rica HPV Vaccine trial (Cervarix) demonstrated that even seven years after vaccination, the efficacy of a single-dose immunization was non-inferior to that of the two- and three dose regimen [125]. This was true for HPV-16 and -18 and also for some non-vaccine types (HPV-31/-33/-45). During the extended time of follow-up, serum antibody levels to HPV-16 and -18 were only 3.7- and 2.6-fold lower in vaccine recipients of a single HPV vaccine dose than in the recipients of three doses. In contrast to differences in the antibody levels, antibody avidity is similar across the recipients of all three types of immunization regimen [134,135]. These findings supported more targeted research on efficacy of the single-dose immunization schedules. One of these clinical trials is currently ongoing: a dose reduction immunobridging and safety study of two HPV vaccines in Tanzanian girls (DoRIS) is aiming to demonstrate non-inferiority of a single dose HPV vaccination schedule compared with the previously approved 2 - 3 dose regimen [136]. In this case, the immunobridging trial represents a post-licensure comparative effectiveness study using vaccine immunogenicity as measure of primary outcome, which is possible due to previously evaluated efficacy and immunogenicity of the full-dose HPV vaccination schedule.

In addition to the focus on dose-reduction, a mixed vaccination schedule was evaluated in the randomized clinical trial setting [93]. This study aimed to exploit and combine advantages of each of the vaccines, such as excellent immunogenicity of the bivalent vaccine and a large number of HPV types targeted by the nonvalent HPV vaccine. The mixed vaccination schedule, such as immunization of the same person with two different HPV vaccines, yielded high anti-HPV antibody responses and demonstrated acceptable safety profile [93]. Flexibility with choosing the type of HPV vaccine for an organized vaccination program would increase market competition in vaccine price and could minimize the prognosed shortage of the HPV vaccines due to the expected widespread launch of vaccination campaigns [137], which are coordinated by the WHO for eliminating cervical cancers worldwide [138].

1.2.3 HPV serology assays for detection of anti-HPV antibodies to viral capsid proteins

In the vast majority of cases HPV infection is cleared within approximately a year. Consequently, studies relying on the detection of cervical HPV DNA often have bias towards underestimation of the total exposure to the pathogen. This was emphasized in the design of HPV vaccination programs when the overall exposure to the virus needs to be estimated in a given population [139]. Compared to the transient HPV infections, anti-HPV antibodies constitute a more stable marker [54]. However, not all of the papillomavirus infections result in a detectable seroconversion [52,54] also explaining why HPV serological assays are used mainly in research but not as a diagnostic tool for cervical cancer screening or triaging [140].

HPV serology assays used in HPV vaccine clinical trials differ in their ability to detect identical subsets of binding and neutralizing antibodies (**Fig. 6**) [141]. Development of multiplexed formats of these serology methods was prompted by emergence of HPV vaccines with multiple genotypes in their formulations [142–146]. Each of these assays has its unique characteristics and advantages/disadvantages, described below and summarized in **Table 1** [147].

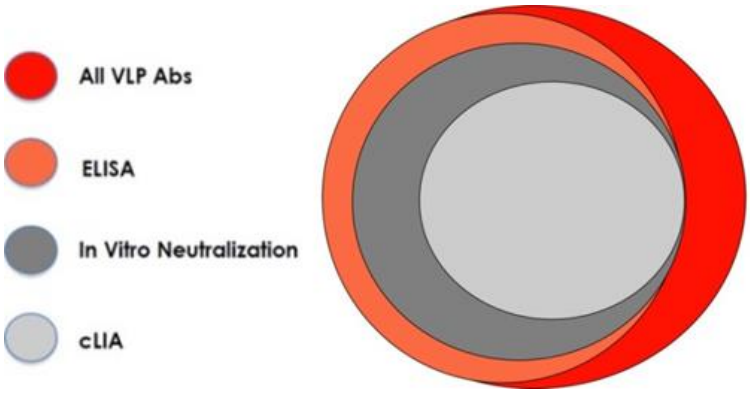

Figure 6. Schematic pattern of the detectable by the serology methods overlapping subsets of binding and neutralizing anti-HPV antibodies against viral capsid proteins. Red designates the total entity of VLP antibodies; orange circle marks subset of antibodies that are detectable by ELISA; grey – by the in vitro neutralizing assay; light grey – by cLIA. cLIA, Competitive Luminex immunoassay; ELISA, Enzyme-linked immunosorbent assay; VLP antibodies, Virus-like particle-specific antibodies [141]. Adapted from Schiller JT, Castellsague X, Garland SM. A review of clinical trials of human papillomavirus prophylactic vaccines. Vaccine. 2012; 30 Suppl 5:F123-38, reproduced with permission from Elsevier, RightsLink Copyright Clearance Center.

Based on the hypothesis that neutralizing anti-HPV antibodies play a key role in protection from HPV infection, the classical method for studying HPV vaccine immunogenicity is the HPV neutralization assay (NA) [97,99,123]. It measures a total subset of neutralizing immunoglobulins (IgM, IgG, IgA). Sensitivity of HPV neutralization assay is similar to that of an enzyme-linked immunosorbent assay (ELISA) but specificity is higher [97].

HPV ELISA is another gold standard used for measuring type-specific antibodies to HPV [148]. It is less laborious than neutralization assays but detects only a certain class of immunoglobulins depending on the class of secondary antibody used [147].

Competitive immunoassay (cLIA) is highly concordant to the neutralization assay [149]. The coefficient of correlation between cLIA and the neutralization assay reaches 0.93 for HPV-16 and 0.95 for HPV-18 [149]. cLIA is also available in a multiplexed format [142].

HPV serology assay	Advantages	Disadvantages
Neutralization assay (NA) / Pseudovirion-based neutralization assay (PBNA)	1. Measures function closest to presumed mechanism of protection through selective detection of neutralizing antibodies 2. All immunoglobulin classes are detected 3. Similar analytic sensitivity compared to a standard VLP ELISA 4. Higher specificity compared to a standard VLP ELISA	1. Requires pseudovirions for each HPV type 2. Requires cell culture and time for cell growth 3. Time consuming, partial automation is possible [99] 4. Multiplexing requires several types of reporter dyes 5. CV is higher than in ELISA
Enzyme-linked immunosorbent assay (ELISA)	1. Faster than the NA 2. Commonly used assay format 3. Multiplexing possible (bead array or multispot wells)	1. Type of secondary antibodies determines a single class of antibodies to be detected (IgG or IgA) 2. Binding antibodies are not be distinguished from the neutralizing
Competitive immunoassay	1. Detects neutralizing antibodies 2. Easily multiplexed with bead arrays (e.g. Luminex) 3. Rapid, high throughput 4. All immunoglobulin classes detected	1. Only a subset of total neutralizing antibodies detected 2. Requires type-specific neutralizing monoclonal antibodies 3. Compromise between choosing the dominant epitope and keeping the type-specificity in a multiplexed format of the assay
Heparin-pseudovirion Luminex assay developed by Helena Faust	1. Fast, high throughput 2. Multiplexed, panel extension possible 3. Scalable 4. Moderately labour intensive when compared to NA and ELISA	1. Type of secondary antibodies determines a single class of antibodies to be detected (IgG or IgA) 2. Binding antibodies are not be distinguished from the neutralizing 3. CV is higher than in HPV VLP ELISA

Table 1. Serology assay types for monitoring HPV vaccine immunogenicity (adapted from [147] and expanded).

The method used in this work for type-specific anti-HPV antibody detection is a multiplexed variant of HPV ELISA, called heparin-pseudovirion Luminex assay [144]. The assay employs pseudovirions of HPV as antigens [70,144]. Aside of being multiplexed, this assay has the advantage of binding of the antigen to the Luminex beads via intermediate layer of heparin, which ensures that only in properly folded capsids are attached [150].

1.2.4 Evaluation of HPV vaccine effectiveness

Estimations of vaccine effectiveness are usually carried out in the post-licensure settings, frequently referred to as “field” settings because study conditions are less controlled than within the clinical trials [151]. In its classical definition, vaccine effectiveness determines reduction in risk of disease among the vaccine recipients relative to unvaccinated. A range of diseases which are caused by the vaccine-targeted pathogen are generally reflected in the spectrum of endpoints for estimating vaccine effectiveness. In case of HPV, it extends also to other measurable responses which serve as proxies for disease protection.

The range of outcomes for evaluation of HPV vaccine effectiveness can be grouped into the disease, virological and immunological endpoints [152]. The use of immunological endpoints as primary outcome measure is so far more suitable for the immunobridging studies, although when applied as a secondary endpoint it is a valuable measure also in the other types of study designs [91,92,101,110,136,153–156]. Relevance of immunogenicity measurements is of limited value as long as a protective antibody threshold is not determined. A second type of endpoints used for estimation of vaccine effectiveness are incident and persistent HPV infections [81,86,89,157]. The last and the most relevant outcomes, the clinical endpoints, reflect course of disease progression from CIN to cervical cancer [82,158–160] and are least immediate to occur. Within the clinical endpoints, however, quadri- and nonavalent vaccines have an advantage since they prevent genital warts and this can be measured shortly after introduction of HPV vaccination. The latter is extremely convenient in evaluation of population level vaccine effectiveness [161–163].

Data on each of the endpoints can be collected in either way – actively through sampling or passively via register-based follow-up in case available infrastructure exists, or through their combination (for instance, retrieval of the biobanked samples). Vaccine effectiveness is measurable both in cohort and case control study design types in their different variants [80,82,90,157,158,164,165].

1.3 MEANS OF CERVICAL CANCER PREVENTION IN SWEDEN

The most effective but somehow unrealistic mean for cervical cancer prevention would be abstinence from sexual intercourses. Luckily, in the era of effective HPV vaccines and cervical screening, this appears to be unnecessary. There are numerous modifications of HPV vaccination and cervical screening programs worldwide. In this thesis, I will focus on Swedish experience.

1.3.1 HPV vaccination

In Sweden, the first HPV vaccine was licensed in 2006 and HPV vaccination became recommended by the Swedish Public Health Agency (Folkhälsomyndigheten, 2007). Initially, the vaccination coverage remained relatively low, barely reaching 33 % and only in a small number of birth cohorts [166]. Coverage of about 80 % was achieved when an organized school-based vaccination program was launched in 2012 [166,167]. Within this program, 10-12 years old girls were vaccinated with three doses of a quadrivalent HPV vaccine. In addition, a catch-up campaign covering girls up to 18 years of age was launched in 2013, and later to young women up to 26 years of age.

Meanwhile, non-inferiority of a two-dose to the previously conventional tree dose vaccination schedule was demonstrated and in 2014 a reduced-dose vaccination regimen was approved by EMA [128]. In 2015, the Swedish national vaccination program was modified to a two-dose girls-only vaccination [168]. In 2019, vaccination was changed to the nonavalent HPV vaccine, and boys were included in August 2020 [167].

The Public Health Agency in Sweden is responsible for the planning and monitoring of the national HPV-vaccination program. There are several ways from monitoring program effectiveness, such as registration of all vaccinations, monitoring and investigation of the infection-related diseases (for instance, by using data collected by The Swedish National Cervical Screening Registry), seroepidemiological surveillance and monitoring of the epidemiology of virus types.

From 2013 on, individual-level records of all vaccinations within the Swedish vaccination programs are collected by **The Swedish National Vaccination Register (NVR)** under the Public Health Agency of Sweden [169]. It contains the following data: vaccination date, personal identifying number (PIN) of the vaccine recipient, type of vaccine being administered, vaccine batch number and the healthcare provider who performs immunization. The place of registration of the vaccine recipient is uploaded from the population register. In addition, person-wise data on HPV vaccinations which might be not covered by the organized immunization program, is collected by **The Swedish Prescribed Drug Register (PDR)**, National Board of Health and Welfare [170]. The PDR was established in 2005 to increase patient safety and it collects data on each dispensation at a pharmacy. PDR data is used by researchers, authorities, analysts etc. and contains information about the patient (sex, age, residence), product (drug code, drug name, strength, inclusion in the pharmaceutical benefits scheme), prescription (quantity/number of packages, strength, date of prescription, date of purchase), costs (total cost, patient cost, region's cost and additional patient cost) and the prescriber.

1.3.2 Cervical screening

Organized cervical cancer screening started in Sweden at the end of the 1960 [171]. It was based on detection of cellular changes and over the following decades reduced notably cervical cancer incidence and mortality (**Fig. 7**). Within this program, women resident in Sweden were invited for cervical sampling either at a three- (23 - 50 years old) or five-year interval (50 - 60 year old) [171].

Within the organized cervical cancer screening program, women resident in Sweden receive invitation letter for attending cervical sampling during the year they turn 23. Participation in the organized screening is free of charge. Today, the program has more elaborated algorithm that utilizes both cytology and HPV-typing as primary screening methods depending on age of the screening participant [171]:

- 23 - 30 years-old women are screened using a liquid cytolgy method at a three-year interval;
- 30 - 49 years-old women are tested for cervical pathology using an HPV DNA detection method (HPV test) also at a three-year interval, with additional cytological test when turning 41;
- 50 - 64 years-old women are screened also with HPV test but only each seventh year;
- women who did not attent sceening at 64 continue receiving annual invitations until they turn 70, and from 70 years on they are released from the program [172].

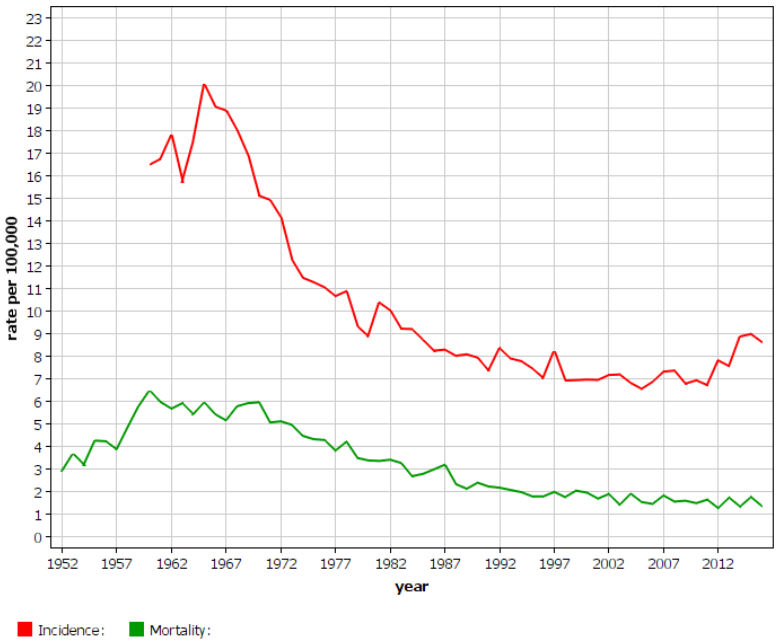

Figure 7. Time trends for age-standardized rates of cervical cancer incidence and mortality in Sweden, all age groups. Curve of the incidence rates has red color and of mortality - green. Visualized with NORDCAN (Nordic tool for cancer information, planning, quality control and research) [173].

Individual-level data about the cervical screening is collected by **The Swedish National Cervical Screening Registry (NKCx)** for monitoring quality and performance of the preventive program [174]. It has a 100 % national coverage and includes all data on issued invitations for cervical screening, on results of cytological and histopathological evaluations. Fine structure of collected data and its completeness make NKCx a valuable source of information for the researches [175–177].

2 AIMS

The overarching aim of this thesis was to estimate effectiveness of HPV vaccination by characterizing duration, breadth and magnitude of vaccine-induced anti-HPV antibody responses and by investigating the reasons for cervical lesion development among the recipients of HPV vaccines. In addition, we aimed to better understand biological characteristics of HPV infection, which prompted us to study dynamics of infection-induced humoral responses to HPV and their relation to sexual activity, positivity to HPV DNA and clinical manifestations. These aims are summarized in **Table 2.**

Paper	Short name	Study aims
I.	Concordance between HPV DNA and anti-HPV antibodies	To characterize anti-HPV antibody response dynamics to a subset of anogenital HPV types and to evaluate their correlation with the cumulative presence of HPV DNA of the same type in the longitudinally collected serial samples.
II.	Infections with multiple HPV types	To assess whether seropositivity to multiple anogenital HPV types is associated with current multiple anogenital HPV infections, abnormal cytology, or seropositivity for cutaneous HPVs.
III.	Long-term immunogenicity of vaccine-targeted types - a head-to-head vaccine comparison	To compare Gardasil- and Cervarix-induced anti-HPV 16 and 18 antibody levels head-to-head up to 12 years post vaccination.
IV.	Long-term immunogenicity of cross-reactive responses - a head-to-head vaccine comparison	To compare Gardasil- and Cervarix-induced seropositivity, antibody levels and antibody avidity for non-vaccine HPV types up to 12 years postvaccination.
V.	Cervical lesions among the HPV vaccinated young women	To investigate whether development of cervical intraepithelial lesions in HPV-vaccinated young women associated with vaccine-targeted HPV types or not.

Table 2. Summary of study aims targeted in each of the papers within the scope of this thesis.

3 FUNDING SOURCES

The work was funded by:

- The Swedish Cancer Society (numbers CAN 2015/399 and CAN 2014/603)
- The Swedish Foundation for Strategic Research (RB13-0011)
- The European Union's Seventh Framework Program for research, Technological Development, and Demonstration, under the CoheaHr Project (HEALTH-F3-2013–603019)
- The Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana;
- The Academy of Finland
- Funding from Karolinska Institutet to M. L. Professorship (Dnr 2–103/2014 and Dnr. 2-3698/2017)
- Travel funding from Karolinska Institutet and from Radiumhemmet.

4 MATERIALS AND METHODS

4.1 STUDY DESIGNS

Papers I and II are based on the same research project and are both longitudinal cohort studies with repeated measurements at two time points (**Fig. 8**). This enabled us to estimate seroconversion rates over the follow-up time, to establish the association between HPV DNA positivity and type-specific anti-HPV antibody levels, to study the effect of infections with multiple HPV types, etc. Measurement from two time points also allowed to estimate protective effect of anti-HPV 16 antibodies induced by HPV infection (not covered by this thesis) [58]. Cohort types of study designs are rated high by the level of epidemiological evidence they can provide. There are, however, certain disadvantages: cohort studies are usually costly and time consuming. In contrast to case-control studies and clinical trial designs, cohort studies may be more subject to bias and are not always appropriate for studying rare events.

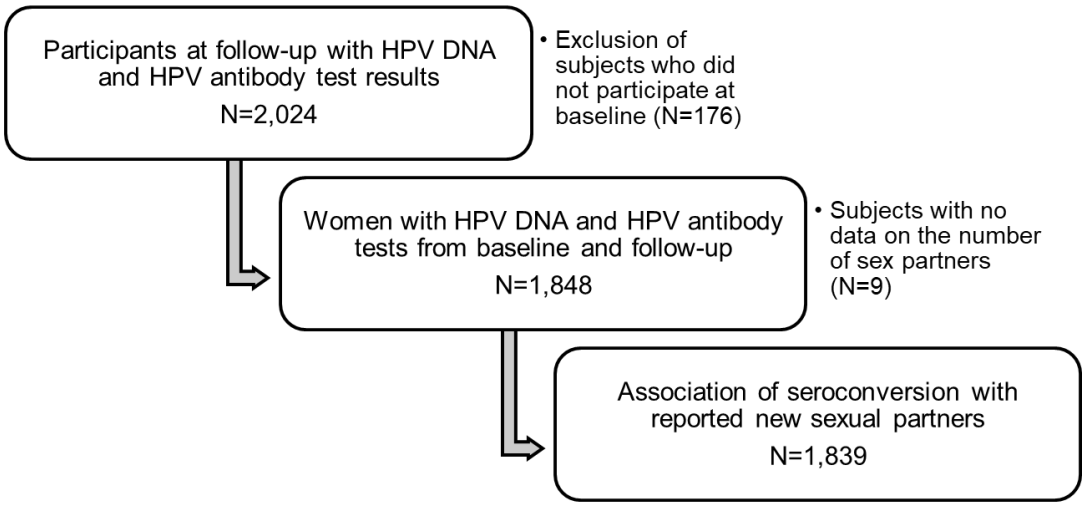

Figure 8. Schematic description of the study design in papers I-II (figure created from [54,178]).

Cervical cancer screening settings appeared to be extremely useful in collecting different types of biological material and study subject information that allowed achieving multiple aims of this study. Repeated measurements of HPV DNA within the same study subject was also helpful in overcoming the documented problem of low sensitivity of HPV serology assays, known to arise from the transient nature of HPV infections.

Papers III and IV arise from one data source and have an identical longitudinal cohort study design, which can also arguably be classified as repeated measurement cross-sectional study. The cohorts were established by the long-term register-based follow-up of women who were earlier enrolled into the one of the phase III clinical trials of HPV vaccines, either Cervarix or quadrivalent Gardasil (**Fig. 9**).

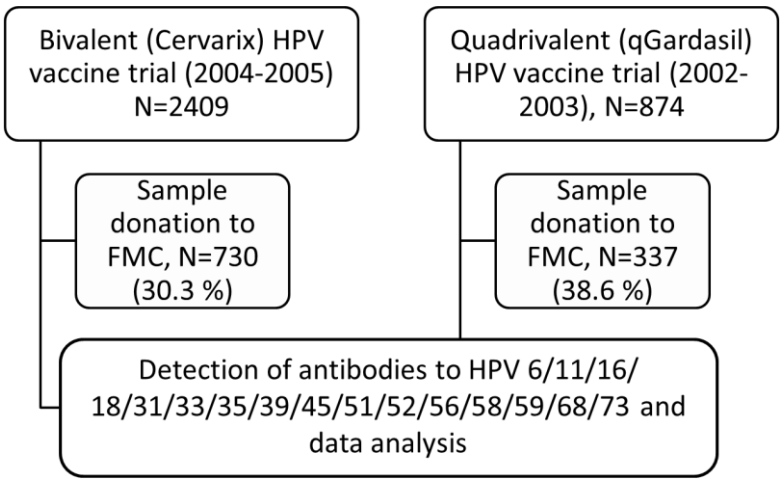

Figure 9. Schematic description of the study design in papers III-IV (figure created from [126]). FMC: Finnish Maternity Cohort.

Paper V has a case-control design, nested among the vaccinated women who attended cervical cancer screening program in Sweden (**Fig. 10**). We excluded women with abnormal cytology/biopsy results before vaccination, abnormal cytology/HPV test that were not confirmed by histology, females with lesions of vagina and women with other types of histological pathologies of cervix. To increase the likelihood of being HPV-naïve by the time of vaccination, we limited the cohort to females immunized at a relatively young age (< 17 years). Sampling location was narrowed to the greater Stockholm area, which represents 23% of the total Swedish population and has high coverage of cervical cancer screening program. The proportion of cervical abnormalities that are routinely detected in cervical screening in the greater Stockholm area is similar to that which is reported by other regions of Sweden. Among the resulting subset of women, rare cases with cervical lesions despite HPV vaccination were matched to the disease-free controls at a 1:2 ratio. Samples from this matched cohort were then retrieved from the cervical cytology biobank for HPV DNA typing.

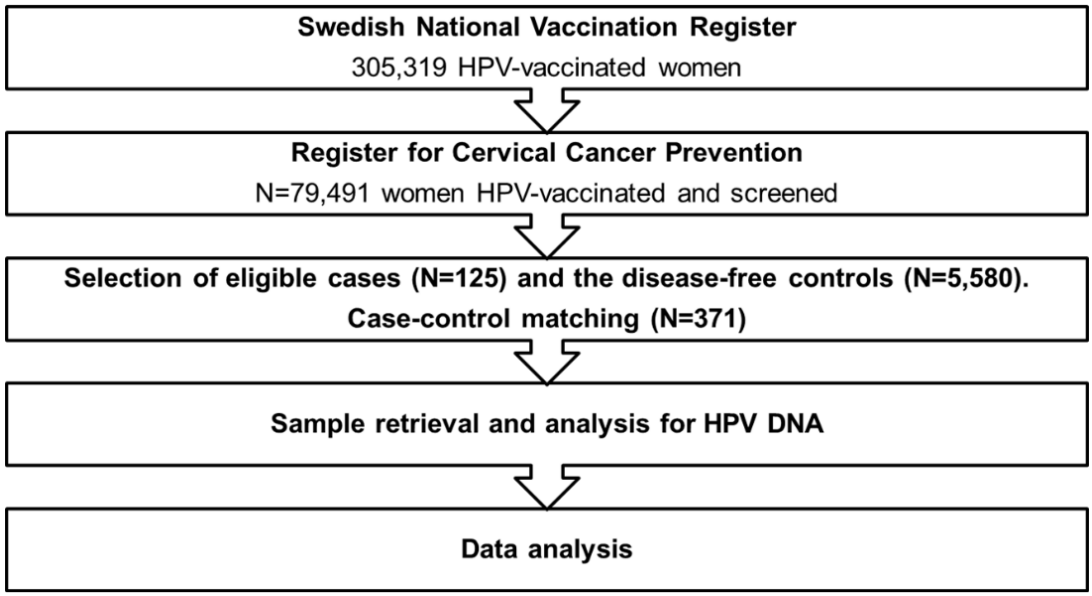

Figure 10. Schematic description of the study design in paper V (figure created from [164]).

Although on the hierarchy of epidemiological evidence this type of study design is less valuable than clinical trials and cohort study designs, case-control studies are in practice

easier to implement when more rare events, such as cervical lesions among young HPV-vaccinated women, shall be analyzed. Further, they are more convenient in studying multiple exposures and are usually less costly than the cohort studies [179].

4.2 STUDY POPULATIONS

In papers I and II, the study population comprised of 2,037 women of 20 - 64 years, who attended cervical cancer screening program in Slovenia and consented for cervical sampling and for blood withdrawal for the anti-HPV antibody testing. These women were also asked to complete the questionnaires about their HPV vaccination status and about a subset of co-factors which are recognized to play role in cervical lesion development. Biological specimens and questionnaires were collected during two consecutive rounds of the study three years apart. Women, who claimed to have been vaccinated with any of the HPV vaccines (N=13), were excluded.

For our research purposes, the data on HPV DNA and anti-HPV antibody status from the two timepoints, as well as cytology results from the second screening round were available for 1,848 women.

In papers III and IV, the study population comprised young women (average age at sampling 27 years), who were vaccinated with three doses of one of the HPV vaccines 7 - 12 years prior to sampling (**Table 3**). At sampling, the women were pregnant and were residing in Finland.

	Bivalent HPV vaccine (Cervarix, N=730)	**Quadrivalent HPV vaccine (Gardasil, N=337)**
Age at vaccination (mean (SD))	17.01 (0.48)	17.07 (0.63)
Age at sampling (mean (SD))	26.50 (1.34)	27.29 (1.62)
Interval from vaccination, years	**N (%)**	
7	63 (8.6)	19 (5.6)
8	114 (15.6)	37 (11.0)
9	149 (20.4)	43 (12.8)
10	208 (28.5)	73 (21.7)
11	185 (25.3)	86 (25.5)
12	11 (1.5)	79 (23.4)
Pregnancy sample serial number	**N (%)**	
1	353 (48.4)	163 (48.4)
2	280 (38.4)	133 (39.5)
3	79 (10.8)	37 (11.0)
4	15 (2.1)	4 (1.2)
>4	3 (0.4)	0 (0.0)

Table 3. Characteristics of study cohorts in the HPV vaccine comparison study (papers III and IV).

At the age of 16 - 17 years, these females were enrolled in one of the clinical trials of HPV vaccines, the bivalent Cervarix (clinical trial PATRICIA) and the quadrivalent Gardasil (clinical trial FUTURE II). The girls consented to a passive follow-up through the Finnish healthcare-related registries and to a subsequent linkage of their personal identifiers with the data in the Finnish Maternity Cohort (FMC) biobank. This allowed the prospective retrieval of serum

samples and a head-to-head comparison of long-term immunogenicity between the two vaccines in the independent settings.

The age-matched cohort of Swedish women who were not vaccinated with any of the HPV vaccines, was used a reference group. Serum samples from these women were obtained from the Region Skåne biobank. Estimates of naturally acquired anti-HPV antibody responses within the nonvaccinated cohort allowed us to compare HPV vaccine-induced cross-reactivity with the HPV seroprevalence resulting from seroconversion to HPV types that circulate in the population.

In paper V, the study population comprised a cohort of young women having been HPV-vaccinated at <17 years of age and attended cervical cancer screening program in Sweden. Selection of eligible cases and a cohort of controls was followed by case-control matching, sample retrieval and HPV DNA typing. 367 women entered the final case-control cohort analysis (**Table 4**). All females were vaccinated with at least one dose of HPV vaccine on average at 16 years of age. Of these, 88 % of cases and 91.3 % of controls received three doses of HPV vaccine. Women who developed cervical neoplasia were on average 23 years old at the time of diagnosis, which corresponds to their first screening round in an organized cervical cancer screening program.

			CIN 1-3
		controls (N=242)	**cases (N=125)**
Vaccine type, n (%)	quadrivalent	240 (99.2 %)	125 (100.0 %)
	quadrivalent and bivalent	2 (0.8 %)	0 (0 %)
Number of doses, n (%)	1	6 (2.5 %)	6 (4.8 %)
	2	15 (6.2 %)	9 (7.2 %)
	3	221 (91.3 %)	110 (88.0 %)
Age at vaccination, years	Mean ± SD	15.79 ± 0.82	15.91 ± 0.78
Age at sampling, years	Mean ± SD	22.68 ± 1.64	22.92 ± 1.68
Interval between vaccination and sampling, years	Mean ± SD	6.89 ± 1.44	7.00 ± 1.51
Age at diagnosis, years	Mean ± SD	-	23.00 ± 1.70

Table 4. Summary characteristics of the HPV-vaccinated case-control cohort which developed CIN1-3 or was free from disease (paper V, adapted from [164]). Reproduced with permission from the John Wiley and Sons, RightsLink Copyright Clearance Center.

4.3 DATA SOURCES

Data collection included both “dry” (register-based) and “wet” (experimental) parts. The second part consisted of test results of sample analyses around the time of their collection (no storage in a biobank) or after retrieval from the biobanks.

In the Slovenian cervical screening cohort study (papers I-II) we used a dataset, which was collected prospectively over the study rounds and contained pseudonymized identifiers, test results of the biospecimen (HPV DNA status, anti-HPV antibody status, cervical cytology result) and responses from the self-reported questionnaires (age, parity, HPV vaccination status, smoking status, number of lifetime partners, number of new male sex partners acquired during the last three years and within the last year). Cervical swabs were used for HPV DNA-testing and for assessment of cytological status (Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Slovenia). The algorithm of HPV DNA typing by the Abbott RealTime High Risk HPV assay, Hybrid Capture 2 HPV DNA assay, Linear Array HPV Genotyping Test, INNO-LiPA HPV Genotyping Extra Test, and an in-house GP5+/GP6+ PCR is described elsewhere [178]. Serum type-specific anti-HPV antibodies were detected at the Karolinska Institute using the heparin-pseudovirion serology assay (Department of Laboratory Medicine, Karolinska Institutet, Sweden) [54,70,144].

In papers III-IV, register-based linkages were followed by the laboratory analyses. Records on the HPV vaccine clinical trials participants (PATRICIA and FUTURE II) that are stored at the Register of Vaccinated Individuals (RVI) were linked to the FMC biobank using the PIN of the former clinical trial participant. This allowed identification of the available biobanked serum samples from the HPV-vaccinated women. Aliquots of these samples were retrieved from the FMC biobank and tested for presence of anti-HPV antibodies using heparin-pseudovirion Luminex assay (Karolinska Institutet). The final dataset that was generated by this study and consisted of the following variables: pseudonymized personal identifier, information from the RVI (type of vaccine, age at first dose of HPV vaccine, vaccination date for each of the received dose), FMC biobank data (pseudonymized sample code, sampling date, serial number of sample/pregnancy) and results of the laboratory analysis (HPV seropositivity status, anti-HPV antibody levels and avidity).

In paper V, in a similar way, data from NVR were linked to the NKCx database by the PIN numbers. This allowed the identification of women who attended cervical screening after having been immunized with any of HPV vaccines. Data on the individual’s personal number, vaccine type, vaccination dates and vaccine dose number were extracted from the NVR and selected PIN were linked to the NKCx. Variables that were used from the NKCx register are: person identifier, age by 2018, sample location county, SNOMED codes for cytology diagnosis, age at diagnosis, date of cytology sample is taken, identification of cytology sample, number of cytology screenings, ID of histopathology diagnosis, date for histopathology sampling etc.

The selection of eligible case-control cohort and status verification among the cases was performed using the subset of SNOMED codes for cervical diseases [180]. The stratification of cases by their CIN grade was based on the uniformed disease classification system. Cervical samples from 125 cases (vaccinated women with CIN1 - 3) and 242 controls were retrieved from the cytology biobank of the Karolinska University Laboratory and analyzed for presence of type-specific HPV DNA. The final dataset, which was used for analysis, contained data on pseudonymized patient ID, sample ID, case-control status, ordinal number for the case-

control strata, grade of cervical neoplasia, type of vaccine, number of doses, vaccination date for each vaccine dose, sampling date, diagnosis date, internal quality control (β-globin copy number), and test results for 37 genital HPV types (HPV DNA positive/negative).

Biobanks

The Finnish Maternity Cohort (FMC) biobank (papers III-IV)

The FMC biobank was established in 1983 for cryopreservation of residual volumes of sera from samples obtained within the congenital disease screening program (first trimester of pregnancy) [181]. Since that time, practically all women having had their pregnancy in Finland have contributed to the biobank collection. FMC register allows for the linkages with the Finnish population-based health registers [182].

The cytology biobank of the Karolinska University Laboratory / National Clinical Cytology Biobank (paper V)

This clinical cytology biobank was established in 2011 as extension of the organized cervical cancer screening program [183]. Today, it collects cytology cell swabs from virtually all gynecological samplings taken within the Stockholm County (about 700,000 samples). The biobank stores cervical cell fractions (600 µl) of liquid-based samples at -25°C for preventing DNA, RNA and proteins degradation, and for maintaining cell morphology. Quality controls of the stored material are performed twice a year.

4.4 LABORATORY METHODS

4.4.1 Multiplex HPV serology assay: heparin-pseudovirion Luminex assay

This technique is based on an antigen-antibody binding similar to classical ELISAs but transformed into a high-throughput method through multiplexing [184]. The assay measures antibodies to multiple HPV types at the same time, which in turn facilitates evaluation of HPV type-specific antibody responses in a large numbers of serum samples. This is achieved with the use of Luminex suspension array technology (xMap): multiple light sources inside the Luminex analyzer excite the internal bead dyes that identify each microsphere particle and any fluorescent reporter molecules captured during the assay (**Fig. 11, B**) [185]. The instrument records dozens of readings for each bead set and produces a distinct result for each analyte in the sample. Using this process, xMAP allows multiplexing of up to 500 unique bioassays within a single well [185]. In the HPV serology multiplex method, beads with different emission profile carry pseudovirions of a specific HPV type (**Fig. 11, B**).

Validation of the method demonstrated a strong type-specific correlation with HPV infection as measured by the cervical HPV DNA positivity [144] was shown to be suitable for studying antibody responses after/during HPV infection and after HPV vaccination [184]. In the heparin-pseudovirion Luminex assay, mammalian cell-derived pseudovirions are used as antigens. The pseudovirions contain both L1 and L2 capsid proteins, which mimic virions but without encapsidated viral genome.

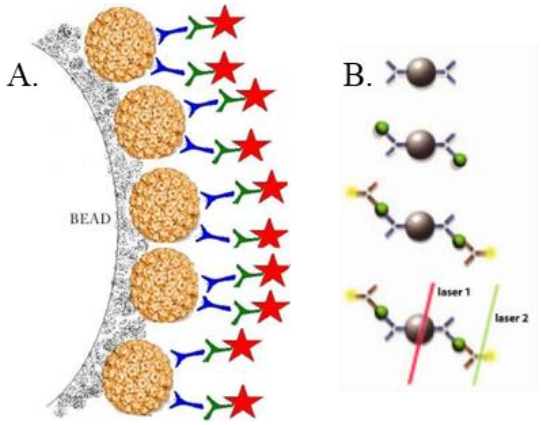

Fig. 11. Heparin-pseudovirion Luminex assay principle [184]. A. Schematic overview of the method. B. Luminex assay principle. On panel A: polystyrene Luminex beads coated with heparin are depicted with gray color; human papillomavirus pseudovirions are marked with orange spherical structures with bound antibodies; human antibodies are depicted with blue color, mouse anti-human – with green and phycoerythrin-tagged goat anti-mouse antibodies are designated with red. On panel B: polystyrene Luminex beads are depicted as gray spheres; two lines of red and light green color represent lasers of Luminex analyzer.

During preparation steps, polystyrene Luminex beads are coated with heparin (gray color on **Fig. 11, A**), which selectively binds properly folded pseudovirions (orange spherical structures on **Fig. 11, A**) [150]. During the sample analysis, pseudovirion-heparin coated beads are incubated with human sera, allowing binding of the anti-HPV antibodies. Wells of the filter plates are washed and the remaining complexes are incubated for one hour with anti-human mouse antibodies. Following washing, phycoerythrin-tagged goat anti-mouse antibodies are added incubated for 30 minutes, which precedes detection of the bound complexes (**Fig. 11, B**). Today, VLPs of at least 23 HPV types, two human polyomavirus types and antigen of HSV2 (gG2 protein) were validated using this method [70,186–188].

The quality of the assay is monitored in an HPV type-specific manner using the negative (sera from children) and positive control panels. The latter consists of monospecific sera and sera from subjects who are seropositive to multiple HPV types. The cut-off for seropositivity is defined by the reactivity of a negative control panel of sera from children six month to twelve years of age (N=±200). Cutoff values of each type of HPV is assigned according to recommendations of the WHO HPV Laboratory Manual (average of MFI values (MFI, median fluorescence intensity) of a negative control serum panel +3 standard deviations) [148]. For HPV-16 and -18, antibody levels are translated into the international units (IU) by calibrating MFI values of the test sample against the reference International Standard (IS) sera established by the WHO. The IS standard sera are provided by the National Institute for Biologicals Standards and Control (NIBSC) [148,189,190].

There are three modes of calibration that can be used for conversion of MFI values into the IU: PLL (parallel line model), wPLL (weighted parallel line model) and RFL (reference line model) [191]. Although the WHO HPV laboratory manual recommends use of the PLL method, higher reproducibility was demonstrated using the RFL model [192]. According to our experience, the latter is indeed more suitable, particularly when using the postvaccination sera which often contains high titres of anti-HPV antibodies. However, for most of the HPV types IS are not yet established. For this reason, we used arbitrary assigned units (AU), defined by measurements from a pool of sera from vaccinated individuals, when investigating antibodies against HPV-6, -11, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -68, and -73, (described in Paper IV).

Avidity, strength of binding between the antigen and antibody, was also measured using the heparin-pseudovirion Luminex assay. The HPV type-specific avidity index (AI) is estimated after antibody-binding following a 10 minute-incubation in 2.5 M ammonium thiocyanate [193]. The AI is defined as a percentage of MFI values in the well which was treated with NH_4SCN compared to identical dilution treated with the PBS only.

4.4.2 High-throughput HPV DNA detection and typing

Initially developed at the German Cancer Research Center [194], the method of multiplexed HPV DNA typing is nowadays used also with the modified general primers (MGP) described elsewhere [194,195].

Multiplexed HPV genotyping is based on PCR products from MGP (or GP5+/6+) PCR, where MGP the reverse primers are biotinylated, and on HPV type-specific probes which are coupled to the polystyrene beads through an amino group (**Fig. 12**) [194,195]. Polystyrene Luminex bead are classified by the color of their inner dye, so that the HPV-type specificity is labeled and is detectable even after mixing the probe-bead solutions for different HPV types. The biotinylated PCR products are then allowed to hybridize to the bead-bound probe. At the next step, streptavidin-R-phycoerythrin binds to the bead-bound biotinylated PCR product, which is then detected by the laser in the Luminex machine. A red laser classifies the beads that are identified by their distinct orange/red emission profile and a green laser detects phycoerythrin bound to the hybridized complexes [196], which allows quantification of the captured PCR product. We used type-specific probes for HPV-6, -11, -16, -18, -18v (a letter that follows the HPV type stands for virus variant), -26, -30, -31, -33, -35, -35v, -39, -40, -42, -43, -45, -51, -51v, -52, -53, -54, -56, -58, -58v, -59, -61, -66, -67, -68a, -68b, -70, -73, -74, -81, -82, -83,- 86, -87, -89, -90 and -91 [164]. The method allows simultaneous detection of at least twice as many HPV types as we used [194].

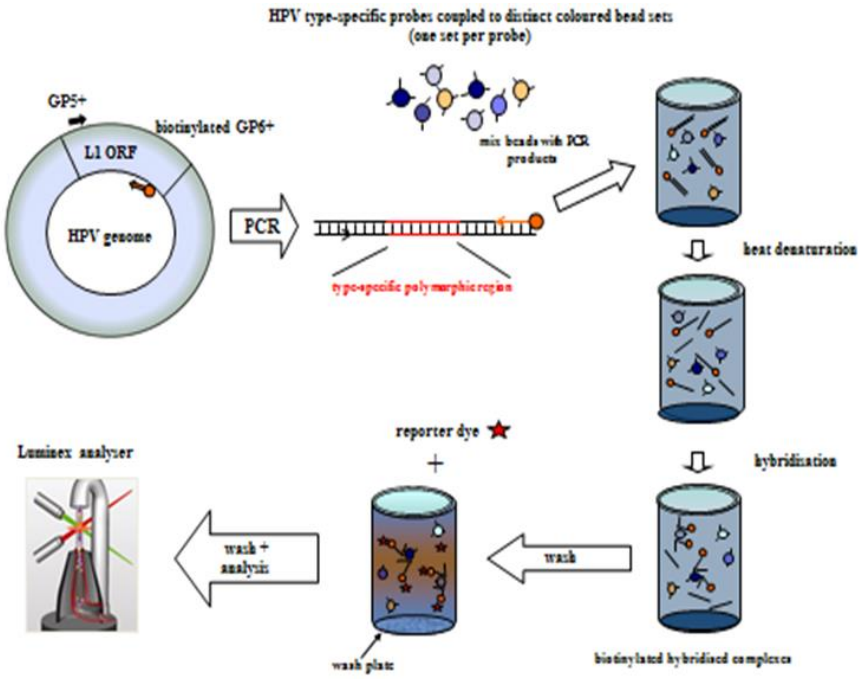

Fig. 12. Schematic overview of multiplex HPV genotyping of GP5+/6+-PCR products using bead-based assay. ORF, open reading frame [194]. Reproduced from Markus Schmitt et al. Bead-Based Multiplex Genotyping of Human Papillomaviruses. J of Clin Microbiol, Feb 2006, 44 (2) 504-512; DOI: 10.1128/JCM.44.2.504-512.2006, with permission from ASM, RightsLink Copyright Clearance Center.

4.5 STATISTICAL METHODS

Variables, estimates, statistical methods and analysis tools used within the research projects of this thesis are summarized in **Table 5**. In papers I and II, we evaluated reproducibility of the serological assay on each of the study rounds by estimating agreement between categorical data (κ statistics) and between numerical (R^2). Less known than R^2, which measures strength of linear association between the variables, κ statistics is based on comparison of the observed proportion of agreement between the two readings, made on two different occasions, with the proportion of agreements that would be expected by chance [197]. In our studies, estimation of sensitivity and specificity of the serological assay relied on the HPV DNA status as gold standard. Due to known transient nature of HPV infections, we estimated serology assay performance with cumulative HPV DNA status from both timepoints. Seroconversion was defined as seropositivity at follow-up among women who had been seronegative at baseline. Strength of association between the HPV DNA/cytology/number of partners etc and anti-HPV antibody statuses were measured by odds ratios (OR), meaning ratio of the odds for becoming antibody positive when, for instance, one is HPV DNA positive compared to the anti-HPV DNA negative.

Paper	Short name	Measured variables	Estimates	Statistical method	Software
I.	Concordance between HPV DNA and anti-HPV antibodies	Cervical HPV DNA status, type-specific anti-HPV antibody status, new sex partner during the last three years	Prevalence and 95 % CI, sensitivity, specificity, seroconversion	Estimates of CI using Wilson/Brown method	GraphPad Prism 7.01
			Categorical data and numerical data agreement	κ statistics, R^2	
			Odds ratio (OR) and 95 % CI	CI estimated with Baptista-Pike method	StatCalc module of Epi Info7
II.	Infections with multiple HPV types *Statistical calculations performed by Dr. Helena Faust*	HPV DNA, cervical cytology, anti-HPV antibody status, anti- PyV antibody status, new sex partner (last three years), lifetime number of partners	Test for trend in proportions	χ2 test for trend	StatCalc module of Epi Info 7
			Odds ratio (OR) and 95 % CI	CI estimated with Baptista-Pike method	
III.	Long-term immunogenicity of vaccine-targeted types - a head-to-head vaccine comparison	Anti-HPV antibody status, anti-HPV antibody levels, years from vaccination	Prevalence and 95 % CI	CI estimated with Wald method	R (version 3.4.3, packages *ggpubr*, *prevalence*, *ggplot2*)
			Comparison of means (non-parametric)	Wilcoxon rank-sum test	

IV.	Long-term immunogenicity of cross-reactive responses - a head-to-head vaccine comparison	Anti-HPV antibody status, anti-HPV antibody levels, antibody avidity, years from vaccination	Prevalence and 95 % CI	CI estimated with Wald method	R (version 3.6.1 packages *reshape2*, *gtools*, *tidyverse*, *RegParallel, tableone, scales*, *ggpubr*, *ggplot2)*
			Proportion comparison	Fisher exact test, $\chi 2$ test for trend in proportions	
			Comparison of means (non-parametric)	Wilcoxon rank-sum test, Kruskal-Wallis test	
			Quantification of fold-differences in antibody levels and in avidity	Linear regression (log-transformed variables)	
V.	Cervical lesions among the HPV vaccinated young women	Exposure: HPV DNA status Outcome: cervical disease status Other variables: number of vaccine doses etc	Odds ratio (OR) and 95 % CI	Conditional logistic regression	R (version 3.5.0, package survival), StatCalc module of EpiInfo (version 7.2.0.1)

Table 5. A summary of statistical methods used in each of the papers within the scope of this thesis.

In papers III and IV, each vaccine recipient group within two-year intervals from vaccination was stratified by the quartiles of their anti-HPV16 antibody levels. This allowed for a more detailed evaluation of seropositivity, anti-HPV antibody levels and avidity of the genital HPV types. Proportions of seropositivity were estimated with Wald confidence intervals. Comparison of seropositivity proportions were carried out with the Fisher exact test (two group comparisons, for instance, vaccine recipient groups) and $\chi 2$ test for trend in proportions (more than two comparison groups, for instance, anti-HPV16 antibody quartile groups). Comparison of median anti-HPV antibody responses and avidity across the groups was as well performed with the non-parametric tests. Wilcoxon rank-sum test was used for testing differences in means across two groups and Kruskal-Wallis – across three or more groups. This was justified by the non-normal distribution of the data and, in some more detailed analyses, by the limited sample sizes.

Differences in means of antibody levels and avidity across the anti-HPV16 antibody quartiles were quantified with linear regression (differences between the log-transformed type-specific anti-HPV antibody levels in each of the anti-HPV antibody quartile group), where the lowest anti HPV-16 quartile group was used as a reference group.

In paper V, logistic regression was used to estimate association between the HPV DNA positivity and the clinical outcome (CIN 1-3) in a case-control study. To control the potential confounding by the number of HPV vaccine doses, we further adjusted our statistical model for the corresponding variable. The other factors which could otherwise influence the association between the exposure and the outcome were initially ruled out in our study design by limiting age at vaccination to 17 years and by matching the cases with the controls

(case-control sets were matched by the age at vaccination, type of HPV vaccine and date of cervical sampling). Estimates for non-vaccine HPV types with less than five events of HPV DNA positivity were not considered for further analysis. HPV types with no positive HPV DNA events in the control groups were evaluated using the StatCalc module of EpiInfo (version 7.2.0.1) because its default algorithm adds 0.5 to each sum of HPV DNA positive and negative women in both case and control groups.

5 ETHICAL CONSIDERATIONS

In the projects in this thesis, we conducted register-based research, laboratory analyses (testing of human blood serum and cervical samples), statistical analyses and prepared the manuscripts, which was all done at Karolinska Institutet. Corresponding research plans were reviewed and approved by the institutional ethical review boards of the local and collaborative parties (**Table 6**). Biological material was obtained from different types of sources and populations. Samples that were used in the studies, were taken either from participants of a cervical cancer screening program or from participants of screening for congenital infections during their first trimester of pregnancy. Informed consent was obtained from study participants prior to sampling. In the research project described in papers I - II, most of the study participants have filled in questionnaires, thus provided additional sensitive information. Key files are handled by the collaborators or securely stored by the local database administrator. Identities of the study participants and their interests are protected by law. Deanonymization of the identifiers is not possible. Pseudonymized identifiers can be traced to the individual only by the authorized personnel and in accordance to the aims stated in the ethical approval of a study.

Paper	**Short name**	**Ethical approvals**
I - II.	I. Concordance between HPV DNA and anti-HPV antibodies. II. Infections with multiple HPV types.	Each round of the study was conducted in accordance with the ethical standards of the Helsinki Declaration and was approved by the National Medical Ethics Committee at the Ministry of Health of the Republic of Slovenia (consent numbers for the baseline and second screening rounds of the study: 83/11/09 and 109/08/12, respectively). Written informed consent was obtained from all study participants. Antibody testing at Karolinska Institutet for detecting anti-HPV antibodies was performed according to permission from the ethical review board (diary number 2016/919-31/2).
III - IV.	III. Long-term immunogenicity of vaccine-targeted types - a head-to-head vaccine comparison. IV. Long-term immunogenicity of cross-reactive responses - a head-to-head vaccine comparison.	The FUTURE II and PATRICIA trials, establishment of registers for HPV vaccinated individuals and their register-based follow-up: approved by the Finnish National Ethical Review Board (ETENE/Tukija) (Dnr's: 52/04/02 and 17/04/04). The FMC-samples were collected, based on informed consent, and maintained for scientific research by Finnish law (number 327/2001:1). Linkage of the intervention registers, the FMC, the FCR and other health registers was approved by the Finnish National Institute for Health & Welfare (THL/1289/5.05.00/2010). Antibody testing at Karolinska Institutet for detecting anti-HPV antibodies and for conducting serological survey of Swedish reference population: approved by the Ethical review board (Dnr 2016/919-31/2). Antibody testing of Slovenian reference population: ethical considerations mentioned in Paper I and II.
V.	V. Cervical lesions among the HPV vaccinated young women.	Study approved by the Stockholm Regional Ethical Review Board (Dnr. 2016/919-31/2). Informed consent from the study participants was not required.

Table 6. Ethical approvals for each of the papers within the scope of this thesis.

In papers I-V, from the preanalytical stages of sample collection or retrieval from the biobanks, and through the following steps of laboratory testing, statistical analysis and manuscript preparation, individual numbers of all study participants were pseudonymized.

In paper V, Swedish women who were vaccinated with any of the HPV vaccines and developed a CIN of any grade were followed up through the registers and were matched with the disease-free vaccinated controls. The ethical review board decided that signed informed consent was not needed from the study participants because of the pseudonymization of personal identifiers and due to the use of archival samples. Register-based research work was conducted with the pseudonymized identifiers. Cervical samples from this case-control cohort were retrieved from the cytology biobank and typed for HPV DNA, with yet another pseudonymization prior to laboratory testing. Disease status was masked from the laboratory personnel until the completion of laboratory analysis and was link to the pseudonymized identifiers prior to statistical analysis step.

In our practical experiments, we used the *E. coli* safety stain XL10-Gold (Ultracompetent Cells) and the immortalized mammalian cell line 293 TT (derivate of human embryonic kidney cells HEK 293, expressing a mutated SV40 large T antigen [98]) to produce antigens for the in-house serology test kits [98,198]. The work was done in a BSL2 laboratory. Safety risks and requirements applicable to handling of human samples were assessed.

None of the projects included animal experiments.

6 MAIN FINDINGS AND DISCUSSION

6.1 NATURALLY ACQUIRED TYPE-SPECIFIC ANTIBODIES MARK HPV INFECTION AND CLINICAL OUTCOME

Determination of the serology assay performance:

We observed that the presence of type-specific serum antibodies is strongly associated with the presence of cervical HPV DNA, showing an average OR of 8.1 for HPV-16, -18, -31, -33, -35, -39, -45, -52, -56, -58, -59. Sensitivity of the serology assay was on average 51.5 % and specificity was 87.1 %.

Baseline seropositivities to multiple genital HPV types are common

At baseline, 65.3 % of women were seropositive to at least one of the 15 anogenital HPV types, being highest for HPV-16 (25.2 %), HPV-58 (18.7 %) and HPV-6 (18.2 %). 22.9 % of women were seropositive for a single HPV type only, while 42.4 % were positive for multiple types. The seroprevalences of the studied non-genital types HPV-3,-5,-15,-32,-38,-76 ranged between 4.8 % and 19.9 %. In serum samples from women seropositive to multiple anogenital HPVs, non-genital types were more likely to be detected than in the other groups, most notably for HPV-32: almost 77 % of women seropositive to HPV-32 belonged to the group of women with multiple anogenital HPV types. The seroprevalences across the polyomaviruses MCPyV and JCPyV were similar (68.8 % and 66.5 %, respectively).

Dynamics of seropositivity to HPV: antibody stability, seroconversion and seroreversion

Seropositivity to most of the anti-HPV antibodies had a high likelihood for persisting over the three years of follow-up: more than 75 %, of women who were positive to anti-HPV-56, -59, -58, -16 and -31 at baseline had detectable antibodies at the follow-up. Positivity to anti-HPV-35, -39 and -45 was least stable, meaning that only 40.3 %, 49.5 % and 52.6 % of baseline seropositive women three years after had detectable antibodies. Seroconversion to HPV-35, -6 and -11 (9.7 %, 10.5 %, and 8.0 %, respectively) occurred more often than to the other types.

When stratifying by the number of HPV types detected serologically within the same individual, most of the study participants over the follow-up time remained in the same group of their antibody status (antibodies to 0, 1 - 2, 3 - 15 HPV types). 75.1 % of women in the group with ≥ 3 HPV types remained seropositive three years later. In group positive to 1 - 2 HPV types, 20.5 % of women seroreverted and became seronegative. In contrast, 35.3 % of initially seronegative women seroconverted by the time of repeated measurement.

Determinants of HPV seropositivity: presence of HPV DNA in the cervix and abnormal cytology

Multiple infections (seropositivity against at least two HPV types) in cumulative serological estimates were detected in 53.3 % of women. Once seropositive, women on average had antibodies to three HPV types.

Compared to the entire study cohort, seropositivity to three or more anogenital HPV types was observed among the women who were younger (median 35 years at baseline) and self-

reported a higher number of lifetime sexual partners (median = 4). At baseline, seropositivity for multiple anogenital HPV types was associated with (i) presence of any HPV DNA at baseline ($\chi2 = 68.8$; $p < 0.0001$), (ii) presence of DNA of ≥2 HPV types at baseline ($\chi2 = 58.6$; $p < 0.0001$), (iii) presence of any HPV DNA at follow-up ($\chi2 = 22.9$; $p < 0.0001$), and (iv) abnormal cytology ($\chi2 = 9.8$; $p = 0.0017$). Baseline antibody positivity for multiple anogenital HPV types did not influence detection of DNA of multiple HPV types at the follow-up ($\chi2 = 2.9$; $p = 0.087$). At follow-up, seropositivity to multiple anogenital HPV types was strongly associated with (i) baseline HPV DNA positivity (any HPV $\chi2 = 111.3$; multiple HPV DNA $\chi2 = 72.9$; all $p < 0.0001$), (ii) any HPV DNA positivity at the follow-up ($\chi2 = 63.9$; $p < 0.0001$) and (iii) multiple HPV DNA positivity at the follow-up ($\chi2 = 11.4$; $p < 0.0008$).

Determinants of HPV seropositivity dynamics: cervical HPV DNA positivity and acquisition of a new sex partner

Seroconversion to HPV-16, -31, -39, -45, -52, -56, -58 and -59 strongly associated with presence of cervical HPV DNA at baseline. HPV DNA positivity among the seronegative women strongly associated with seroconversion at follow-up, in particular for HPV-58 (OR=46.2; 95 % CI: 8.3-257.0) and HPV-39 (OR=24.9; 95 % CI: 7.3-84.8). Acquisition of a new sex partner associated with seroconversion for HPV-6, -16, -33, -35 and -56 (OR=1.8; 95 % CI: 1.3-2.6; OR=1.8; 95 % CI: 1.2-2.6; OR=1.9; 95 % CI: 1.2-2.9; OR=1.5; 95 % CI: 1.0-2.1; OR=2.0; 95 % CI: 1.2-3.2, respectively).

Compared to women with a decreasing number of HPV type-specific antibodies, seroconversion to 1 or more types of HPV was associated with HPV DNA positivity at baseline (OR= 2.0; 95 % CI 1.23-3.34; p= 0.0046), at follow-up (OR= 2.6; 95 % CI 1.43-4.65; p= 0.0013), or with a self-reported new sex partner (OR= 1.5; 95 % CI 1.07-2.22; p=0.0221). In this type of analysis other parameters did not associate with HPV antibody dynamics.

Summary

Collectively, we found that characteristics of type-specific anti-HPV antibodies to the most common oncogenic HPV types (HPV 16 and 18) apply also to the other HR HPVs. In particular, serum antibodies to HPV (i) associate with cervical HPV DNA positivity in a type-specific manner; (ii) stable over time; (iii) appear with a certain delay and, (iv) when using cervical HPV DNA as standard, average sensitivity of detection is around 50 %, which is similar to that of HPV 16. In addition, (v) serum antibodies to multiple anogenital HPV types associate with presence of HPV DNA and with abnormal cytology, (vi) presence of antibodies to anogenital HPVs associate with presence of non-genital HPVs and (vii) seropositivity to multiple HPV types was highly stable over time. Our results on HPV DNA in serial cervical swabs further showed to be valuable in validation of the serology assay as repeated sampling for exposure may improve sensitivity without impairing specificity.

Discussion

We demonstrated that the cumulative measurements improve the single point estimates. However, improvement in pseudovirion-Luminex assay sensitivity were to some extend papillomavirus species-dependent when measurements of HPV DNA positivity at both time points were used instead of the single timepoint data. The reasons for this phenomenon are not known.

The study has several unique strengths in type of design, compliance of study cohort, range of methods that were employed and in its overall timeliness. In addition, detection of serological responses to HPV infection and testing for the HPV infection were done with validated assays. This was important for evaluation of type-specific association between the anti-HPV antibodies and HPV DNA infection. Heparin-pseudovirion Luminex serology assay has the widest panel of the antigens yet described in the literature.

There are also some weaknesses in our study, such as a relatively large number of women (N = 32) who have had their cytology results within the "gray zone" (classified as atypical squamous cells of undetermined significance). We have added these cases to the sub-cohort with abnormal results, assuming the worse direction of the outcome, although it could introduce some bias towards the null hypothesis (no association between seropositivity to multiple HPV types and cervical lesions). Another limitation is relatively limited number of HPV types for studying multiple infections. In addition, at follow-up we could not evaluate performance of HPV-68 because the different subtypes of this genotype were used in the HPV DNA primers used for identifying infections and in the L1 sequence of plasmid construct used for serology antigen production. This may have resulted in detection of anti-HPV antibodies against subtype 68a, while the detected infections were caused by the subtype 68b.

Despite the decline of HPV DNA prevalence, anti-HPV antibodies are detectable in the populations at a more stable rate. Possible reasons for seroconversion to multiple HPV types include specific features of infection (exposures to multiple antigens), as well as biological properties of the host (certain women are more likely to elicit anti-HPV antibodies than the other females). Data supporting the hypothesis of a "good seroconverter" phenotype further appears in the study of anti-HPV antibody responses among the vaccinated (paper IV).

6.2 HPV VACCINES INDUCE STABLY HIGH AND CROSS-REACTIVE ANTI-HPV ANTIBODY RESPONSES EVEN 12 YEARS AFTER IMMUNIZATION

Anti-HPV antibody responses to vaccine-targeted HPV types 7-12 years from immunization are stably high in the BVR and the QVR

A total of 92.3 % of QVR and 100 % of BVR had anti-HPV16 antibody levels above those induced after HPV infection. Anti-HPV18 seroprevalence was lower in the QVR compared to the BVR (81.9 % of vs 99.8 %, $p < 0.0001$). Notably, 7 - 12 years post vaccination anti-HPV16 and -18 antibody levels induced by each of the vaccines were stable and remained well above the median seropositivity levels of natural infection. The avidities of antibodies to vaccine-targeted HPV types 16 and 18 were also higher in BVR.

Anti-HPV-16 and HPV-18 antibody levels were substantially above the median anti-HPV antibody levels of natural infection: median anti-HPV-16 antibody levels were on average 14.8 times higher among the QVR and 73.3 times higher among the BVR. Similar estimates for HPV-18 were on average 5.1 and 80.0 times higher for the QVR and BVR, respectively.

Throughout 7 - 12 years post vaccination, median antibody levels of both HR vaccine-targeted HPV types differed between bivalent and quadrivalent vaccines (average p-value <0.0001). Compared to QVR, recipients of Cervarix maintained on average 5.1 (HPV-16) and 18.4 (HPV-18) times higher anti-HPV antibody levels.

As expected, seroprevalence of HPV-6 and -11 among the BVR were similar to those of unvaccinated individuals. Approximately ten years after vaccination, 91.7 % and 92.9 % of QVR, and 26.7 % and 14.1 % of BVR were seropositive to the vaccine-targeted low-risk HPV types. Antibody levels and avidity were also lower in the BVR.

Bivalent and quadrivalent HPV vaccines induce broadly cross-reactive and long-lasting anti-HPV antibody responses

Seroprevalence to non-vaccine HPV types (HPV-31, -35, -45, -51, -52, -56, -58, and -68) were higher in the BVR than QVR. Compared to non-vaccinated females matched by age, seropositivity to non-vaccine HPV types was generally also higher among the vaccinated women.

In both vaccine cohorts, seropositivity to non-vaccine types increases with increase in the HPV-16 antibody levels

Women were grouped by the anti-HPV-16 antibody levels and by the vaccine type. Among the recipients of the bivalent HPV vaccine, anti-HPV-16 antibody levels and type-specific avidity to HPV-16 and -18 were elevated in the highest quartiles of anti-HPV-16 antibody levels ($p<0.01$). Likewise, antibody levels to the non-vaccine types 31, 35, 51, 52 and 58 were higher in the upper levels of anti-HPV-16 antibodies ($p<0.01$ for each one of these non-vaccine type). In a similar manner, seropositivity also increased with the anti-HPV-16 titer, most remarkably for types 31, 33, 35, 45, 51, 52, 58, 59, 68, and 73 (for each of these types: $\chi 2$ test $p\leq 0.001$). Levels of non-vaccine types HPV31, 35, 51, 52, 56 and 58 also increased in the upper quartiles of anti-HPV16 antibody levels. Antibody avidity did not differ across the strata of the anti-HPV-16 antibody titers.

Among the recipients of the quadrivalent HPV vaccine, seroprevalence of the vaccine-targeted types HPV-6, -11, -16 and -18 increased when grouped by the anti-HPV-16 antibody levels (test for trend in proportions $p<0.001$). In line with this, antibody levels of HPV-6, -11 and -18 increased in the upper quartiles when compared to the lower strata of anti-HPV-16 antibody levels ($p<0.001$ for each of the listed types). When compared to the lowest quartiles of anti-HPV16 antibody levels, anti-HPV-6, -11 and -16 antibody avidity increased in each subsequent anti-HPV-16 antibody level strata. This was the case also with the seroprevalence of the non-vaccine HPV types, most notably for HPV-31, -33, -35, -45, -52, -58, -59, and -68 (for each of these HPV types: $\chi 2$ test $p\leq 0.001$). Levels of non-vaccine types did not increase in the upper quartiles of anti-HPV16 antibody levels when compared to the lowest. Like in the BVR, antibody avidity did not differ in the QVR across the strata of the anti-HPV16 antibody titers.

Vaccine-induced seroprevalence, but not antibody levels and avidity, are higher in the BVR than in the QVR

In all strata of anti-HPV-16 antibody levels, seroprevalence to HPV-31, -35 and -58 were higher in the BVR compared to the QVR ($p <0.005$ for each type). Proportions of vaccine recipients who were seropositive to HPV-45, -51 and -52 were also higher among the BVR than in the QVR but this was not consistent across the different strata of anti-HPV-16 antibody levels. In vaccine recipients with low (below median) anti-HPV-16 antibody levels, BVR had higher proportions of seropositive individuals than the QVR, most notably for HPV-31, -35, -52 and -58 ($p\leq 0.001$ for each type). In vaccine recipients with high (above median) anti-HPV16

antibody levels, the seroprevalences to an even larger number of HPV types (p≤0.001 for -31, -35, -45, -51, -58; p=0.02 for HPV-52) were higher in the BVR than in the QVR.

Anti-HPV16 antibody levels appeared to have little effect on differences in the antibody levels of the other HPV types across the vaccine recipient cohorts. Irrespectively of the anti-HPV16 antibody strata, anti-HPV6/11 antibody prevalence, levels and avidity were higher among the QVR compared to BVR.

Anti-HPV antibody levels in BVR detected shortly after HPV vaccination correlate with anti-HPV antibody levels measured a decade later

Anti-HPV antibody levels for vaccine types HPV-16 and -18 measured shortly after immunization, predicted antibody levels 7 - 12 years later. A subset of the BVR (N=96) were sampled also shortly after HPV vaccination, at seven-month interval (M7) after the first vaccine dose. This subset was stratified by the quartiles of the anti-HPV-16 and -18 antibody levels at M7. Antibody levels 7 - 12 years later were compared in the second, third and fourth quartiles vs the first quartile of M7 antibody level strata. BVR in the upper HPV-16 and -18 quartiles, about ten years later had up to three-fold higher antibody levels to HPV-16 and -18, respectively, when compared to those in the lowest antibody quartiles (p <0.001). In a similar way, short-term antibody levels predicted avidity levels for HPV-16 (1.5 - 1.8 times higher, p≤0.01) but not for HPV-18.

Summary

In an independent, population-based setting, we followed-up women for up to twelve years from vaccination. We found that: (i) antibody levels induced by Cervarix were stable and even twelve years after vaccination virtually all women had anti-HPV antibody levels above those induced by the HPV infection; (ii) most of the women who were vaccinated with Gardasil had long-lasting stable anti-HPV antibody levels which, however, declined below the antibody level of natural infection in 8 % (HPV-16) and in 18 % (HPV-18) of the QVR; (iii) anti-HPV-16 antibody levels were 5.1-fold higher and anti-HPV-18 were 18.4-fold higher in the BVR compared to QVR (p <0.0001). Also, (iv) seropositivity to most of the non-vaccine HPV types was more common among the BVR than the QVR; (v) anti-HPV antibody levels and avidity were comparable between the vaccines for almost all HPV types; (vi) vaccine-induced seroprevalence to HPV-31, -33, -35, -45, -52, -58, -59, -68 and -73 increased with anti-HPV-16 levels; (vii) among the BVR, levels of non-vaccine types HPV-31, -35, -51, -52, -56 and -58 also increased in the upper quartiles of anti-HPV-16 levels; (viii) anti-HPV antibody levels at month 7 predicted antibody levels 10 years later.

Discussion

In our study, 82 % of QVR had their anti-HPV-18 antibody levels above those which are acquired due to infection. Our findings of the cross-reactive antibody responses to HPV-31, -33, -45, -52, and -58 are in line with those found in a slightly shorter follow-up study [118]. Compared to this similar study, we found lower proportions of women seropositive to the non-vaccine HPV types [118]. This might at least partially be explained by natural decline in the antibody levels over the long time. We also observed that seropositivity to HPV-52 and -58 was more common among the BVR than among the QVR (p<0.001). Data on these two HPV types were inconsistent in previous reports and a meta-analysis resulted in no significant difference between the vaccines [122].

Our preliminary data using a neutralization assay are largely similar to the results observed with the current HPV antibody binding assay [120]. The few exceptions are higher seroprevalence of anti-HPV6 and anti-HPV33 antibodies in the neutralization assay. Otherwise, seroprevalence estimates for HPV types 31, 45, 52, and 58 measured with the neutralization assay were quite similar to those detected with our antibody binding assay.

In our previous studies of anti-HPV antibody responses in the non-vaccinated cohort, some women appeared to seroconvert particularly well to HPV infections, suggesting that a high responder subgroup of women might exist [178]. Current findings in the vaccinated cohort support our earlier observations with the fact that women with higher anti-HPV antibody levels to vaccine-targeted HPV16 have also apparent higher seropositivity to almost all non-vaccine HPV types at the same time, irrespectively of HPV vaccine used.

Across the two vaccine recipient cohorts, serological signatures of anti-HPV antibody levels differed between the vaccines, such as only the BVR in the upper quartiles of anti-HPV-16 antibody levels had higher titers to the non-vaccine types HPV-31, -35, -51, -52, -58 and -73 compared to the group with the lowest anti-HPV-16 antibody levels. Our observation are in agreement with the short-term follow-up data on vaccine-targeted and cross-neutralizing types HPV-31, -33, -45, -52, and -58 published by others [117]. As in the neutralization assays, the cross-reactivity pattern was more pronounced among the BVR than the QVR.

The main strengths of this study are its head-to-head comparison setting which allowed for a follow-up of anti-HPV antibody levels in a large cohort of vaccinated women (337 QVR and 730 BVR) and for the longest so far reported interval (seven to twelve years after vaccination). Moreover, we used a validated anti-HPV antibody detection assay, which is independent of the vaccine antigens and comprise a wide panel of HPV types. We also evaluated anti-HPV antibody avidity and converted antibody levels into the International Units. The latter will facilitate future comparisons across the laboratories, methods and HPV types.

There are, however, some limitations. Our vaccine recipient cohort was vaccinated at around 17 years of age, which is higher than recommended and may in turn result in lower antibody levels as compared to vaccination at a younger age [132]. The cohorts differed in their sample size and number of participants had a skewed distribution over the follow-up years. There could have been a selection bias since only pregnant women entered the cohorts. In addition, participants of the PATRICIA vaccine trial were more represented than the FUTURE II (30.3 % vs 38.6 %; $p<0.0001$). Also, we do not know whether pregnancy affects serum antibody levels. Since the recipients of the two HPV vaccines were sampled in identical setting, it would not explain the serological differences observed between the cohorts of vaccinated women.

Currently, clinical significance of seroreversion and of individual-level decline of anti-HPV antibody levels are under evaluation. Linkages to the healthcare registers will allow to identify almost all HPV-associated lesions that might have occurred among the vaccinated women.

6.3 CERVICAL LESIONS AMONG WOMEN IMMUNIZED WITH HPV VACCINES ARE VERY RARELY ASSOCIATED WITH THE VACCINE-TARGETED HIGH-RISK HPV TYPES

Study population

According to the register-based data, 305,320 women have received ≥ 1 dose of HPV vaccine from its licensure in 2006 to 2014. Of these, 26 % attended cervical cancer screening program

in 2007-2016 and have had screening results reported to the NKCx. The following exclusion criteria were further applied to this cohort of women having been both HPV immunized and screened for cervical pathology: abnormal cytology test result or at least one referral to the biopsy prior to HPV vaccination, abnormal cytology that was not confirmed by histological testing, vaginal lesions, and other than CIN1 - 3 cervical diagnoses. In the remaining cohort (N=69,328), 3,576 women were diagnosed with CIN1 - 3. Irrespectively of cervical status, 5,874 were vaccinated at young age (<17 years old) and resided in the greater Stockholm region (CIN1=179, CIN2=69, and CIN3=46) at the time of cervical screening. For these women, the number of vaccine doses was verified using data from the Swedish Prescribed Drug Register (National Board of Health and Welfare).

Cervical samples (N=125: 84 CIN1, 25 CIN2, and 16 CIN3) of 42.5 % of all eligible CIN cases were identified at cytology biobank of the Karolinska University Laboratory. These were matched with eligible disease-free controls from the same cohort of women at a 1:2 ratio. Cryo-preserved cervical samples of interest were retrieved from the biobank, followed by DNA extraction, PCR and Luminex HPV typing. For sample quality reasons, four disease-free women were excluded from the statistical analysis.

HPV prevalence among young vaccinated women differ from HPV DNA prevalence in the historical non-vaccinated cohort

All samples (N=367) were negative for HPV-6 and -11. Compared to the population-based HPV DNA prevalence of vaccine-targeted types in the pre-vaccination time [199,200], we observed much lower HPV DNA positivity proportions in the HPV vaccinated young women. In the pre-vaccination era about 38 % of CIN2+ lesions were HPV-16-positive, while in our study of the immunized cohort, none of samples from women with CIN2-3 contained HPV-16 DNA. However, a subset of the non-vaccine types (HPV-33, -51, -52, -56, -59, and -66) turned out to be more prevalent in the post-vaccination swabs. For example, HPV-39, -42, -68 and -90 positivity (CIN1 only) was more common than in the pre-vaccination time. In women with CIN2-3, HPV-31, -35 and -45 were more prevalent among the vaccinated women than the historical nonvaccinated cohort.

Association between HPV DNA positivity and development of cervical lesions in the young vaccinated women: pattern changes after introduction of HPV vaccination

The pattern of HPV type-specific odds for development of CIN among young women also differed from that of the pre-vaccination time. In the young vaccinated women, HPV DNA positivity for types 45, 51 and 52 associated with the development of CIN2-3, while positivity for HPV-42, -51, -52, -56 and -66 associated with CIN1. HPV DNA positivity to certain HPV types, e.g., HPV-42, -51 and -66, tended to associate stronger with the presence of CIN1 in comparison to the pre-vaccination estimates.

Summary

We found that cervical lesions in women, vaccinated at <17 years of age, usually did not associate with the HPV types targeted by the HPV vaccine but rather with the non-vaccine HPV types.

The main strengths of this study are its population-based design and the use healthcare register data to identify the HPV vaccinated women who have attended the cervical cancer

screening program and to trace the biobanked cervical smears of interest. We assessed cervical HPV DNA status in 42.5 % of all eligible samples from the HPV vaccinated young women who were diagnosed with CIN1 - 3 in the Stockholm region. Most of the diagnoses were revealed 2014 - 2016, when the screening coverage of the youngest age group was the highest across the other age groups. Moreover, HPV DNA was detected and typed by the HPV reference laboratory and using a well-established assay. The effect of HPV vaccination at a relatively young age was investigated within the subsequently detected cervical lesions when vaccine recipients attended their first cervical screening. By reducing our case-control cohort to the young recipients of HPV vaccines (<17 years at vaccination), we were aiming to limit the probability of exposure to HPV infection prior to vaccination.

Of the main limitations of the study are its modest sample size of the cohort of cases, which prompted us for a combined analysis of the group of women who developed CIN2-3 with no further stratification by the risk of lesion progression. Another drawback is the representativeness: the biobank cohort was covering samples only from organized cervical screening, which does not start until women turn 23 years of age. This means that the samples taken at younger age out of the organized cervical screening program are not covered by this study. This, however, in our opinion makes the findings more generalizable to the overall female population.

Population-level decline in HPV DNA prevalence [81,87,201] and cervical disease [159] were observed after introduction of HPV vaccination programmes. Compared to the historical cohort data in the Swedish population [201] and the pre-vaccination meta-analysis data [202], we now observed a remarkable reduction in HPV16/18 prevalence among the disease-free HPV vaccinated young women and those with cervical lesions. These findings are in agreement with vaccine effectiveness found in Scotland by monitoring of an immunization program that was based on the bivalent HPV vaccine showing that the prevalence of low-grade cytological abnormalities was increasing with a concurrent decrease in detection of histological findings [159].

The implications of our findings are that the neoplasms after HPV vaccination are most likely caused by those virus types which are not included in the HPV vaccine. This important limitation is important to communicate for preventing unjustified dissatisfaction with the HPV vaccines to vaccine producers, physicians but also women. However, yet another important message is that most of the HPV types, found in cervical lesions among the vaccinated women, have limited progressive potential (42, 51, 56 and 66).

7 CONCLUSIONS

The results of the presented studies contribute to our understanding of serological responses to HPV infections and vaccination, and can at least partially explain the nature of cervical lesions among young HPV-vaccinated women:

In our studies of serological responses to HPV infection (paper I and II), HPV DNA strongly associated with the type-specific antibodies to HPV-16, -18, -31, -33, -35, -39, -45, -52, -56, -58, and -59. Biological characteristics of serological responses were in agreement with those reported previously for HPV 16 [63]. In addition to these reports, we demonstrated that seropositivity to multiple anogenital HPVs, detectable in a sub-cohort of women, has specific biological characteristics as e.g. tendency to persist over time and strong association with past of current HPV infection, with abnormal cytology and with seroconversion to non-genital HPV types. Overall, our antibody detection against multiple HPV types was extensively validated in the non-vaccinated cohort and was thus found to be useful in the subsequent research projects on the humoral immune responses to HPV infection and HPV vaccination (papers III and IV).

In our studies of vaccine-induced antibody responses we compared the two HPV vaccines in the head-to-head long-term follow-up settings. We demonstrated that even twelve years after immunization, most of the vaccinated women had detectable anti-HPV-16 and anti-HPV-18 antibodies. The antibody levels were well above those induced by the HPV infection, as measured in our earlier studies. However, the antibody levels differed over time across the vaccine recipient cohorts in favor of the bivalent HPV vaccine as a notable fraction of the quadrivalent vaccine recipients lost measurable HPV-18 antibodies during follow-up. Nonetheless, both vaccines induced cross-reactive anti-HPV antibody responses and congruent with our previous observations in the non-vaccinated cohort, some HPV vaccinated women seroconvert particularly well. We further observed that cross-reactive HPV responses were more likely to occur in the vaccine recipients with high anti-HPV 16 antibody responses, which was more frequent in the bivalent vaccine recipients.

The type-specific disease-protective threshold of anti-HPV antibodies is not known but essential for predicting vaccination success. In our next study (paper V), we thus focused on non-serological evaluation of the vaccine effectiveness against the clinical outcomes.

We evaluated potential reasons for development of cervical lesions among young HPV vaccine recipients and found that cervical intraepithelial lesions among women having been HPV-vaccinated at a young age, are very rarely associated with the vaccine-targeted HPV types of high oncogenic risk. This has profound implications for the future design of cervical screening strategies that would target highly vaccinated cohorts of women and for design of surveillance and monitoring strategies, such as opting for less frequent screening starting at older age and monitoring effectiveness using more solid endpoints. Important public health message to the healthcare professionals and vaccinated populations is that cervical lesions may occur despite HPV vaccination. However, these neoplasms are likely caused by HPV types that rarely progress to cancer.

8 OPEN QUESTIONS

Cervical cancer screening and HPV vaccination programs are walking hand-in-hand. That is why from the early time of HPV vaccine development it has been hypothesized that, should HPV vaccines prove efficacious, the introduction of HPV vaccination programs would demand modifications of the ongoing cervical cancer screening programs [26]. The need for this optimization is apparent today, in particular among the highly vaccinated cohorts [176], however a few questions closely related to the effectiveness of HPV vaccines remain unanswered.

One of the most urgent issue is evaluation of a single-dose HPV vaccination regimen [136]. The need for this has been prompted by apparent win in the costs for vaccination and simplification of vaccine delivery, as well as by the emerging fear of HPV vaccine shortage after the WHO call towards the global elimination of cervical cancer. An additional question is whether all three HPV vaccines which are licensed to date would demonstrate non-inferiority of the single-dose application. Should a single-dose vaccination demonstrate non-inferiority to the two-dose schedule and become adopted in the routine vaccination practice, a continuous monitoring of effectiveness of the reduced-dose vaccination programs will be needed, where defined protective level of anti-HPV antibodies would increase effectiveness and speed of the surveillance. Global efforts in cervical cancer elimination accompanied by the efficient surveillance measures also require established international sera standards for all high-risk HPV types and at least two low risk genotypes (HPV-6 and -11) to allow for the direct comparison of the serology assays and for continuous control of their quality.

Evaluation of protective anti-HPV antibody threshold not only against HPV infections but also against the clinical outcomes in needed. Searching for the protective level is challenging because cases of HPV-associated cervical disease among the HPV vaccinated women are uncommon, it usually takes several years to develop the lesion and, once occurring, the disease are mostly associated with the non-vaccine HPV types [164]. *In vivo* murine challenge models suggest that antibody protectivity levels could be at the limit of detection by the currently available serology assays [100]. On the other hand, anti-HPV antibody levels among the HPV-vaccinated HIV-infected subjects were shown to be almost identical in the breakthrough cases and in those women who remained protected [203], urging for search of additional correlates for more successful seroconversion and determinants for protection.

Efficacy and effectiveness studies of HPV vaccines have demonstrated that a certain level of cross-protection against the phylogenetically related non-vaccine HPV types exists and that it differs across the HPV vaccines and HPV types [78,84,85,111]. We have observed that seroprevalence to the non-vaccine types increases with the anti-HPV antibody levels of the vaccine-targeted type. Once high titers of anti-HPV antibodies indeed confer better protection than responses with low levels of antibodies, evaluation of cross-protective potential of vaccine-targeted types would gain utmost practical importance. In addition, inferring from our findings that antibody levels as early as seven months from vaccination at least partially define anti-HPV antibody titers a decade later, evaluation of antibody level soon after vaccination with regard to protective potential decades later would be of clinical significance.

The ongoing evaluations of HPV vaccine effectiveness take place in the populations with continued circulation of the papillomaviruses. Exposure to native antigens may stimulate

immune system in addition to the post-vaccination priming, thus may contribute towards maintenance of high levels of anti-HPV antibodies and broader cross-reactivity. However, it is yet unknown to which extend vaccine-induced antibody responses might be affected should the prevalence of circulating antigens approach zero.

Detection of anti-HPV antibodies in the vaccinated individuals has mainly been performed in the serum samples and less commonly in the cervicovaginal secretions [92,94,95]. Both options are suboptimal when studying serological responses shortly after vaccination: blood withdrawal is invasive and cervicovaginal secretion sampling is not suitable for early adolescent girls. In contrast, detection of anti-HPV antibodies in oral mucosa would overcome these drawbacks, however currently available serology assays will require validation with a new sample type and probably with additional normalization against the total IgG or IgA levels. New findings will continuously refill the list of open questions, and with that I thank you for showing interest to my work!

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10 REFERENCES

1. Doorbar J, Quint W, Banks L, et al. The Biology and Life-Cycle of Human Papillomaviruses. Vaccine. **2012**; 30:F55–F70.

2. Lehtinen M, Hakama M, Aaran RK, et al. Herpes simplex virus type 2 infection and cervical cancer: a prospective study of 12 years of follow-up in Finland. Cancer Causes Control. **1992**; 3(4):333–338.

3. IARC. Human papillomaviruses. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. **2007**; 90.

4. Egawa N, Doorbar J. The low-risk papillomaviruses. Virus Research. **2017**; 231:119–127.

5. Bouvard V, Baan R, Straif K, et al. A review of human carcinogens - Part B: biological agents. The Lancet Oncology. **2009**; 10(4):321–322.

6. Arbyn M, Castellsague X, Sanjose S de, et al. Worldwide burden of cervical cancer in 2008. Ann Oncol. **2011**; 22(12):2675–86.

7. Martel C de, Plummer M, Vignat J, Franceschi S. Worldwide burden of cancer attributable to HPV by site, country and HPV type. Int J Cancer. **2017**; 141(4):664–670.

8. Martel C de, Georges D, Bray F, Ferlay J, Clifford GM. Global burden of cancer attributable to infections in 2018: a worldwide incidence analysis. The Lancet Global Health. **2020**; 8(2):e180–e190.

9. IARC, WHO. Data analysis and visualization tool for studying cancers attributable to infections [Internet]. Available from: https://gco.iarc.fr/causes/infections/tools-map?mode=3&sex=2&continent=0&agent=8&cancer=0&key=asr&scale=quantile

10. Fields BN, Knipe DM, Howley PM, Griffin DE. Fields virology. Philadelphia: Lippincott Williams & Wilkins; 2007.

11. Villiers E-M de, Fauquet C, Broker TR, Bernard H-U, Hausen H zur. Classification of papillomaviruses. Virology. **2004**; 324(1):17–27.

12. The International Human Papillomavirus (HPV) Reference Center [Internet]. Available from: https://www.hpvcenter.se/human_reference_clones

13. International Committee on Taxonomy of Viruses (ICTV) [Internet]. Available from: https://talk.ictvonline.org/taxonomy

14. Muhr LSA, Eklund C, Dillner J. Towards quality and order in human papillomavirus research. Virology. **2018**; 519:74–76.

15. Stanley MA. Epithelial Cell Responses to Infection with Human Papillomavirus. Clin Microbiol Rev. **2012**; 25(2):215–222.

16. Tommasino M. The human papillomavirus family and its role in carcinogenesis. Seminars in Cancer Biology. **2014**; 26:13–21.

17. Borruto F, De Ridder M, editors. HPV and Cervical Cancer. New York, NY: Springer New York; 2012.

18. Surviladze Z, Dziduszko A, Ozbun MA. Essential Roles for Soluble Virion-Associated Heparan Sulfonated Proteoglycans and Growth Factors in Human Papillomavirus Infections. PLOS Pathogens. **2012**; 8(2):e1002519.

19. Kines RC, Thompson CD, Lowy DR, Schiller JT, Day PM. The initial steps leading to papillomavirus infection occur on the basement membrane prior to cell surface binding. PNAS. **2009**; 106(48):20458–20463.

20. Dziduszko A, Ozbun MA. Annexin A2 and S100A10 Regulate Human Papillomavirus Type 16 Entry and Intracellular Trafficking in Human Keratinocytes. J Virol. **2013**; 87(13):7502–7515.

21. Woodham AW, Da Silva DM, Skeate JG, et al. The S100A10 subunit of the annexin A2 heterotetramer facilitates L2-mediated human papillomavirus infection. PLoS ONE. **2012**; 7(8):e43519.

22. Scheffer KD, Gawlitza A, Spoden GA, et al. Tetraspanin CD151 Mediates Papillomavirus Type 16 Endocytosis. J Virol. **2013**; 87(6):3435–3446.

23. Spoden G, Freitag K, Husmann M, et al. Clathrin- and Caveolin-Independent Entry of Human Papillomavirus Type 16—Involvement of Tetraspanin-Enriched Microdomains (TEMs). PLOS ONE. **2008**; 3(10):e3313.

24. Schelhaas M, Shah B, Holzer M, et al. Entry of Human Papillomavirus Type 16 by Actin-Dependent, Clathrin- and Lipid Raft-Independent Endocytosis. PLOS Pathogens. **2012**; 8(4):e1002657.

25. Aydin I, Weber S, Snijder B, et al. Large Scale RNAi Reveals the Requirement of Nuclear Envelope Breakdown for Nuclear Import of Human Papillomaviruses. PLOS Pathogens. **2014**; 10(5):e1004162.

26. Schiller JT, Lowy DR, Markowitz LE. 16 - Human papillomavirus vaccines. In: Plotkin SA, Orenstein WA, Offit PA, editors. Vaccines (Sixth Edition). London: W.B. Saunders; 2013. p. 235–256.

27. Doorbar J, Egawa N, Griffin H, Kranjec C, Murakami I. Human papillomavirus molecular biology and disease association. Reviews in Medical Virology. **2015**; 25:2–23.

28. Krawczyk E, Suprynowicz FA, Liu X, et al. Koilocytosis: A Cooperative Interaction between the Human Papillomavirus E5 and E6 Oncoproteins. The American Journal of Pathology. **2008**; 173(3):682–688.

29. Satterwhite CL, Torrone E, Meites E, et al. Sexually Transmitted Infections Among US Women and Men: Prevalence and Incidence Estimates, 2008. Sexually Transmitted Diseases. **2013**; 40(3):187–193.

30. Woodman CB, Collins S, Winter H, et al. Natural history of cervical human papillomavirus infection in young women: a longitudinal cohort study. Lancet. **2001**; 357(9271):1831–6.

31. Rositch AF, Koshiol J, Hudgens MG, et al. Patterns of persistent genital human papillomavirus infection among women worldwide: A literature review and meta-analysis. International Journal of Cancer. **2013**; 133(6):1271–1285.

32. Woodman CBJ, Collins SI, Young LS. The natural history of cervical HPV infection: unresolved issues. Nat Rev Cancer. **2007**; 7(1):11–22.

33. Winer RL, Xi LF, Shen Z, et al. Viral load and short-term natural history of type-specific oncogenic human papillomavirus infections in a high-risk cohort of midadult women. Int J Cancer. **2014**; 134(8):1889–98.

34. Taylor S, Bunge E, Bakker M, Castellsague X. The incidence, clearance and persistence of non-cervical human papillomavirus infections: a systematic review of the literature. BMC Infect Dis. **2016**; 16:293.

35. Jaisamrarn U, Castellsague X, Garland SM, et al. Natural history of progression of HPV infection to cervical lesion or clearance: analysis of the control arm of the large, randomised PATRICIA study. PLoS One. **2013**; 8(11):e79260.

36. Ho GY, Bierman R, Beardsley L, Chang CJ, Burk RD. Natural history of cervicovaginal papillomavirus infection in young women. N Engl J Med. **1998**; 338(7):423–8.

37. Ramanakumar AV, Naud P, Roteli-Martins CM, et al. Incidence and duration of type-specific human papillomavirus infection in high-risk HPV-naive women: results from the control arm of a phase II HPV-16/18 vaccine trial. BMJ Open. **2016**; 6(8):e011371.

38. Stanley M. Immune responses to human papillomavirus. Vaccine. **2006**; 24, Supplement 1:S16–S22.

39. Ostör AG. Natural history of cervical intraepithelial neoplasia: a critical review. Int J Gynecol Pathol. **1993**; 12(2):186–192.

40. Castle PE, Schiffman M, Wheeler CM, Solomon D. Evidence for Frequent Regression of Cervical Intraepithelial Neoplasia-Grade 2. Obstet Gynecol. **2009**; 113(1):18–25.

41. Nayar R, Wilbur DC. The Pap Test and Bethesda 2014. ACY. **2015**; 59(2):121–132.

42. Sundstrom K, Eloranta S, Sparen P, et al. Prospective study of human papillomavirus (HPV) types, HPV persistence, and risk of squamous cell carcinoma of the cervix. Cancer Epidemiol Biomarkers Prev. **2010**; 19(10):2469–78.

43. Forman D, Martel C de, Lacey CJ, et al. Global Burden of Human Papillomavirus and Related Diseases. Vaccine. **2012**; 30:F12–F23.

44. Doorbar J, Griffin H. Refining our understanding of cervical neoplasia and its cellular origins. Papillomavirus Research. **2019**; 7:176–179.

45. Stern PL, Burg SH van der, Hampson IN, et al. Therapy of Human Papillomavirus-Related Disease. Vaccine. **2012**; 30, Supplement 5:F71–F82.

46. Scott ME, Ma Y, Farhat S, Moscicki A-B. Expression of nucleic acid-sensing Toll-like receptors predicts HPV16 clearance associated with an E6-directed cell-mediated response. International journal of cancer Journal international du cancer. **2015**; 136(10):2402–2408.

47. Hibma MH. The Immune Response to Papillomavirus During Infection Persistence and Regression. Open Virol J. **2012**; 6:241–248.

48. Daud II, Scott ME, Ma Y, Shiboski S, Farhat S, Moscicki A-B. Association between toll-like receptor expression and human papillomavirus type 16 persistence. International Journal of Cancer. **2011**; 128(4):879–886.

49. Stanley M. Immunology of HPV Infection. Curr Obstet Gynecol Rep. **2015**; 4(4):195–200.

50. Fu Y, Cao R, Schäfer M, et al. Expression of different L1 isoforms of Mastomys natalensis papillomavirus as mechanism to circumvent adaptive immunity. Stanley M, Rath S, Christensen N, Stanley M, editors. eLife. **2020**; 9:e57626.

51. Tummers B, Goedemans R, Pelascini LP, et al. The interferon-related developmental regulator 1 is used by human papillomavirus to suppress NFkappaB activation. Nat Commun. **2015**; 6:6537.

52. Carter JJ, Koutsky LA, Hughes JP, et al. Comparison of Human Papillomavirus Types 16, 18, and 6 Capsid Antibody Responses Following Incident Infection. Journal of Infectious Diseases. **2000**; 181(6):1911–1919.

53. Geijersstam V af, Kibur M, Wang Z, et al. Stability over Time of Serum Antibody Levels to Human Papillomavirus Type 16. Journal of Infectious Diseases. **1998**; 177(6):1710–1714.

54. Artemchuk H, Triglav T, Oštrbenk A, Poljak M, Dillner J, Faust H. Seroprevalences of Antibodies to 11 Human Papillomavirus (HPV) Types Mark Cumulative HPV Exposure. J Infect Dis. **2018**; 218(3):398–405.

55. Onda T, Carter JJ, Koutsky LA, et al. Characterization of IgA response among women with incident HPV 16 infection. Virology. **2003**; 312(1):213–221.

56. Gruijl TD de, Bontkes HJ, Walboomers JM, et al. Immunoglobulin G responses against human papillomavirus type 16 virus-like particles in a prospective nonintervention cohort study of women with cervical intraepithelial neoplasia. J Natl Cancer Inst. **1997**; 89(9):630–8.

57. Arnheim Dahlstrom L, Andersson K, Luostarinen T, et al. Prospective seroepidemiologic study of human papillomavirus and other risk factors in cervical cancer. Cancer Epidemiol Biomarkers Prev. **2011**; 20(12):2541–50.

58. Triglav T, Artemchuk H, Oštrbenk A, et al. Effect of naturally acquired type-specific serum antibodies against human papillomavirus type 16 infection. J Clin Virol. **2017**; 90:64–69.

59. Malik ZA, Hailpern SM, Burk RD. Persistent Antibodies to HPV Virus-Like Particles Following Natural Infection Are Protective Against Subsequent Cervicovaginal Infection with Related and Unrelated HPV. Viral Immunology. **2009**; 22(6):445–449.

60. Castellsague X, Naud P, Chow SN, et al. Risk of newly detected infections and cervical abnormalities in women seropositive for naturally acquired human papillomavirus type 16/18 antibodies: analysis of the control arm of PATRICIA. J Infect Dis. **2014**; 210(4):517–34.

61. Viscidi RP, Schiffman M, Hildesheim A, et al. Seroreactivity to Human Papillomavirus (HPV) Types 16, 18, or 31 and Risk of Subsequent HPV Infection: Results from a Population-Based Study in Costa Rica. Cancer Epidemiology Biomarkers & Prevention. **2004**; 13(2):324–327.

62. Beachler DC, Jenkins G, Safaeian M, Kreimer AR, Wentzensen N. Natural Acquired Immunity Against Subsequent Genital Human Papillomavirus Infection: A Systematic Review and Meta-analysis. J Infect Dis. **2016**; 213(9):1444–54.

63. Ho GYF, Studentsov YY, Bierman R, Burk RD. Natural History of Human Papillomavirus Type 16 Virus-Like Particle Antibodies in Young Women. Cancer Epidemiol Biomarkers Prev. **2004**; 13(1):110–116.

64. Vaccarella S, Franceschi S, Clifford GM, et al. Seroprevalence of antibodies against human papillomavirus (HPV) types 16 and 18 in four continents: the International Agency for Research on Cancer HPV Prevalence Surveys. Cancer Epidemiol Biomarkers Prev. **2010**; 19(9):2379–88.

65. Velentzis LS, Sitas F, O'Connell DL, et al. Human papillomavirus 16/18 seroprevalence in unvaccinated women over 30 years with normal cytology and with high grade cervical abnormalities in Australia: results from an observational study. BMC Infect Dis. **2014**; 14:3861.

66. Tiggelaar SM, Lin MJ, Viscidi RP, Ji J, Smith JS. Age-Specific Human Papillomavirus Antibody and Deoxyribonucleic Acid Prevalence: A Global Review. Journal of Adolescent Health. **2012**; 50(2):110–131.

67. Shin HR, Lee DH, Herrero R, et al. Prevalence of human papillomavirus infection in women in Busan, South Korea. Int J Cancer. **2003**; 103(3):413–21.

68. Kovaleva A, Alberts CJ, Waterboer T, et al. A cross-sectional study on the concordance between vaginal HPV DNA detection and type-specific antibodies in a multi-ethnic cohort of women from Amsterdam, the Netherlands - the HELIUS study. BMC Infect Dis. **2016**; 16(1):502.

69. Paaso AE, Louvanto K, Syrjanen KJ, et al. Lack of type-specific concordance between human papillomavirus (HPV) serology and HPV DNA detection in the uterine cervix and oral mucosa. J Gen Virol. **2011**; 92(Pt 9):2034–46.

70. Faust H, Jelen MM, Poljak M, Klavs I, Učakar V, Dillner J. Serum antibodies to human papillomavirus (HPV) pseudovirions correlate with natural infection for 13 genital HPV types. Journal of Clinical Virology. **2013**; 56(4):336–341.

71. Tachezy R, Hamsikova E, Hajek T, et al. Human papillomavirus genotype spectrum in Czech women: correlation of HPV DNA presence with antibodies against HPV-16, 18, and 33 virus-like particles. J Med Virol. **1999**; 58(4):378–86.

72. Group FIIS, Dillner J, Kjaer SK, et al. Four year efficacy of prophylactic human papillomavirus quadrivalent vaccine against low grade cervical, vulvar, and vaginal intraepithelial neoplasia and anogenital warts: randomised controlled trial. BMJ. **2010**; 341:c3493.

73. https://www.ema.europa.eu/en/medicines/human/EPAR/gardasil.

74. https://www.ema.europa.eu/en/medicines/human/EPAR/gardasil-9.

75. https://www.ema.europa.eu/en/medicines/human/EPAR/cervarix.

76. Lehtinen M, Paavonen J, Wheeler CM, et al. Overall efficacy of HPV-16/18 AS04-adjuvanted vaccine against grade 3 or greater cervical intraepithelial neoplasia: 4-year end-of-study analysis of the randomised, double-blind PATRICIA trial. Lancet Oncol. **2012**; 13(1):89–99.

77. Huh WK, Joura EA, Giuliano AR, et al. Final efficacy, immunogenicity, and safety analyses of a nine-valent human papillomavirus vaccine in women aged 16-26 years: a randomised, double-blind trial. Lancet. **2017**; 390(10108):2143–2159.

78. Lehtinen M, Lagheden C, Luostarinen T, et al. Ten-year follow-up of human papillomavirus vaccine efficacy against the most stringent cervical neoplasia end-point-registry-based follow-up of three cohorts from randomized trials. BMJ Open. **2017**; 7(8):e015867.

79. Benard VB, Castle PE, Jenison SA, et al. Population-Based Incidence Rates of Cervical Intraepithelial Neoplasia in the Human Papillomavirus Vaccine Era. JAMA Oncol. **2017**; 3(6):833–837.

80. Kjaer SK, Nygård M, Dillner J, et al. A 12-Year Follow-up on the Long-Term Effectiveness of the Quadrivalent Human Papillomavirus Vaccine in 4 Nordic Countries. Clin Infect Dis. **2018**; 66(3):339–345.

81. Kavanagh K, Pollock KG, Cuschieri K, et al. Changes in the prevalence of human papillomavirus following a national bivalent human papillomavirus vaccination programme in Scotland: a 7-year cross-sectional study. The Lancet Infectious Diseases. **2017**; 17(12):1293–1302.

82. Luostarinen T, Apter D, Dillner J, et al. Vaccination protects against invasive HPV-associated cancers. Int J Cancer. **2018**; 142(10):2186–2187.

83. Garland SM, Hernandez-Avila M, Wheeler CM, et al. Quadrivalent Vaccine against Human Papillomavirus to Prevent Anogenital Diseases. N Engl J Med. **2007**; 356(19):1928–1943.

84. Brown DR, Kjaer SK, Sigurdsson K, et al. The Impact of Quadrivalent Human Papillomavirus (HPV; Types 6, 11, 16, and 18) L1 Virus-Like Particle Vaccine on Infection and Disease Due to Oncogenic Nonvaccine HPV Types in Generally HPV-Naive Women Aged 16–26 Years. J Infect Dis. **2009**; 199(7):926–935.

85. Wheeler CM, Castellsagué X, Garland SM, et al. Cross-protective efficacy of HPV-16/18 AS04-adjuvanted vaccine against cervical infection and precancer caused by non-vaccine oncogenic HPV types: 4-year end-of-study analysis of the randomised, double-blind PATRICIA trial. The Lancet Oncology. **2012**; 13(1):100–110.

86. Donken R, King AJ, Bogaards JA, Woestenberg PJ, Meijer CJLM, Melker HE de. High Effectiveness of the Bivalent Human Papillomavirus (HPV) Vaccine Against Incident and Persistent HPV Infections up to 6 Years After Vaccination in Young Dutch Women. The Journal of Infectious Diseases. **2018**; 217(10):1579–1589.

87. Garland SM, Cornall AM, Brotherton JML, Wark JD, Malloy MJ, Tabrizi SN. Final analysis of a study assessing genital human papillomavirus genoprevalence in young Australian women, following eight years of a national vaccination program. Vaccine. **2018**; 36(23):3221–3230.

88. Latsuzbaia A, Arbyn M, Tapp J, et al. Effectiveness of bivalent and quadrivalent human papillomavirus vaccination in Luxembourg. Cancer Epidemiology. **2019**; 63:101593.

89. Woestenberg PJ, King AJ, Benthem BHB van, et al. Bivalent Vaccine Effectiveness Against Type-Specific HPV Positivity: Evidence for Cross-Protection Against Oncogenic Types Among Dutch STI Clinic Visitors. J Infect Dis. **2018**; 217(2):213–222.

90. Tabrizi SN, Brotherton JML, Kaldor JM, et al. Assessment of herd immunity and cross-protection after a human papillomavirus vaccination programme in Australia: a repeat cross-sectional study. The Lancet Infectious Diseases. **2014**; 14(10):958–966.

91. Draper E, Bissett SL, Howell-Jones R, et al. A randomized, observer-blinded immunogenicity trial of Cervarix((R)) and Gardasil((R)) Human Papillomavirus vaccines in 12-15 year old girls. PLoS One. **2013**; 8(5):e61825.

92. Einstein MH, Levin MJ, Chatterjee A, et al. Comparative humoral and cellular immunogenicity and safety of human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine and HPV-6/11/16/18 vaccine in healthy women aged 18-45 years: follow-up through Month 48 in a Phase III randomized study. Hum Vaccin Immunother. **2014**; 10(12):3455–65.

93. Gilca V, Sauvageau C, Panicker G, De Serres G, Ouakki M, Unger ER. Immunogenicity and safety of a mixed vaccination schedule with one dose of nonavalent and one dose of bivalent HPV vaccine versus two doses of nonavalent vaccine – A randomized clinical trial. Vaccine. **2018**; 36(46):7017–7024.

94. Petäjä T, Pedersen C, Poder A, et al. Long-term persistence of systemic and mucosal immune response to HPV-16/18 AS04-adjuvanted vaccine in preteen/adolescent girls and young women. International Journal of Cancer. **2011**; 129(9):2147–2157.

95. Schwarz TF, Kocken M, Petaja T, et al. Correlation between levels of human papillomavirus (HPV)-16 and 18 antibodies in serum and cervicovaginal secretions in girls and women vaccinated with the HPV-16/18 AS04-adjuvanted vaccine. Hum Vaccin. **2010**; 6(12):1054–61.

96. Scherpenisse M, Mollers M, Schepp RM, et al. Detection of systemic and mucosal HPV-specific IgG and IgA antibodies in adolescent girls one and two years after HPV vaccination. Hum Vaccin Immunother. **2013**; 9(2):314–21.

97. Pastrana DV, Buck CB, Pang YY, et al. Reactivity of human sera in a sensitive, high-throughput pseudovirus-based papillomavirus neutralization assay for HPV16 and HPV18. Virology. **2004**; 321(2):205–16.

98. Buck CB, Pastrana DV, Lowy DR, Schiller JT. Efficient intracellular assembly of papillomaviral vectors. J Virol. **2004**; 78(2):751–7.

99. Sehr P, Rubio I, Seitz H, et al. High-throughput pseudovirion-based neutralization assay for analysis of natural and vaccine-induced antibodies against human papillomaviruses. PLoS One. **2013**; 8(10):e75677.

100. Longet S, Schiller JT, Bobst M, Jichlinski P, Nardelli-Haefliger D. A Murine Genital-Challenge Model Is a Sensitive Measure of Protective Antibodies against Human Papillomavirus Infection ▿. J Virol. **2011**; 85(24):13253–13259.

101. Einstein MH, Baron M, Levin MJ, et al. Comparative immunogenicity and safety of human papillomavirus (HPV)-16/18 vaccine and HPV-6/11/16/18 vaccine: follow-up from months 12-24 in a Phase III randomized study of healthy women aged 18-45 years. Hum Vaccin. **2011**; 7(12):1343–58.

102. Kemp TJ, García-Piñeres A, Falk RT, et al. Evaluation of Systemic and Mucosal Anti-HPV16 and 18 Antibody Responses from Vaccinated Women. Vaccine. **2008**; 26(0):3608–3616.

103. Olsson S-E, Villa LL, Costa RLR, et al. Induction of immune memory following administration of a prophylactic quadrivalent human papillomavirus (HPV) types 6/11/16/18 L1 virus-like particle (VLP) vaccine. Vaccine. **2007**; 25(26):4931–4939.

104. Nygard M, Saah A, Munk C, et al. Evaluation of the Long-Term Anti-Human Papillomavirus 6 (HPV6), 11, 16, and 18 Immune Responses Generated by the Quadrivalent HPV Vaccine. Clin Vaccine Immunol. **2015**; 22(8):943–8.

105. Hoes J, Pasmans H, Knol MJ, et al. Persisting Antibody Response 9 Years After Bivalent Human Papillomavirus (HPV) Vaccination in a Cohort of Dutch Women: Immune Response and the Relation to Genital HPV Infections. J Infect Dis. **2020**; 221(11):1884–1894.

106. Roteli-Martins CM, Naud P, De Borba P, et al. Sustained immunogenicity and efficacy of the HPV-16/18 AS04-adjuvanted vaccine: up to 8.4 years of follow-up. Hum Vaccin Immunother. **2012**; 8(3):390–7.

107. Naud PS, Roteli-Martins CM, De Carvalho NS, et al. Sustained efficacy, immunogenicity, and safety of the HPV-16/18 AS04-adjuvanted vaccine: final analysis of a long-term follow-up study up to 9.4 years post-vaccination. Hum Vaccin Immunother. **2014**; 10(8):2147–62.

108. Ferris D, Samakoses R, Block SL, et al. Long-term study of a quadrivalent human papillomavirus vaccine. Pediatrics. **2014**; 134(3):e657-65.

109. Schwarz TF, Galaj A, Spaczynski M, et al. Ten-year immune persistence and safety of the HPV-16/18 AS04-adjuvanted vaccine in females vaccinated at 15-55 years of age. Cancer Med. **2017**; 6(11):2723–2731.

110. Einstein MH, Takacs P, Chatterjee A, et al. Comparison of long-term immunogenicity and safety of human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine and HPV-6/11/16/18 vaccine in healthy women aged 18-45 years: end-of-study analysis of a Phase III randomized trial. Hum Vaccin Immunother. **2014**; 10(12):3435–45.

111. Malagón T, Drolet M, Boily M-C, et al. Cross-protective efficacy of two human papillomavirus vaccines: a systematic review and meta-analysis. The Lancet Infectious Diseases. **2012**; 12(10):781–789.

112. Bogaards JA, Weele P van der, Woestenberg PJ, Benthem BHB van, King AJ. Bivalent Human Papillomavirus (HPV) Vaccine Effectiveness Correlates With Phylogenetic Distance From HPV Vaccine Types 16 and 18. J Infect Dis. **2019**; 220(7):1141–1146.

113. Carozzi F, Puliti D, Ocello C, et al. Monitoring vaccine and non-vaccine HPV type prevalence in the post-vaccination era in women living in the Basilicata region, Italy. BMC Infectious Diseases. **2018**; 18(1):38.

114. Lehtinen M, Luostarinen T, Vänskä S, et al. Gender-neutral vaccination provides improved control of human papillomavirus types 18/31/33/35 through herd immunity: Results of a community randomized trial (III). International Journal of Cancer. **2018**; 143(9):2299–2310.

115. Barzon L, Squarzon L, Masiero S, et al. Neutralizing and cross-neutralizing antibody titres induced by bivalent and quadrivalent human papillomavirus vaccines in the target population of organized vaccination programmes. Vaccine. **2014**; 32(41):5357–62.

116. Herrin DM, Coates EE, Costner PJ, et al. Comparison of adaptive and innate immune responses induced by licensed vaccines for human papillomavirus. Human Vaccines & Immunotherapeutics. **2014**; 10(12):3446–3454.

117. Mariz FC, Bender N, Anantharaman D, et al. Peak neutralizing and cross-neutralizing antibody levels to human papillomavirus types 6/16/18/31/33/45/52/58 induced by bivalent and quadrivalent HPV vaccines. npj Vaccines. **2020**; 5(1):1–6.

118. Godi A, Panwar K, Haque M, et al. Durability of the neutralizing antibody response to vaccine and non-vaccine HPV types 7 years following immunization with either Cervarix® or Gardasil® vaccine. Vaccine. **2019**; 37(18):2455–2462.

119. Folschweiller N, Behre U, Dionne M, et al. Long-term Cross-reactivity Against Nonvaccine Human Papillomavirus Types 31 and 45 After 2- or 3-Dose Schedules of the AS04-Adjuvanted Human HPV-16/18 Vaccine. J Infect Dis. **2019**; 219(11):1799–1803.

120. Mariz et al. Sustainability of neutralizing antibodies induced by bi- or quadrivalent HPV vaccines correlates with efficacy. tLID. **2020**; Submitted.

121. Toh ZQ, Kosasih J, Russell FM, et al. Selective Persistence of HPV Cross-Neutralising Antibodies following Reduced-Dose HPV Vaccine Schedules. Vaccines. **2019**; 7(4):200.

122. Bissett SL, Godi A, Jit M, Beddows S. Seropositivity to non-vaccine incorporated genotypes induced by the bivalent and quadrivalent HPV vaccines: A systematic review and meta-analysis. Vaccine. **2017**; 35(32):3922–3929.

123. Faust H, Toft L, Sehr P, et al. Human Papillomavirus neutralizing and cross-reactive antibodies induced in HIV-positive subjects after vaccination with quadrivalent and bivalent HPV vaccines. Vaccine. **2016**; 34(13):1559–1565.

124. Villa LL, Ault KA, Giuliano AR, et al. Immunologic responses following administration of a vaccine targeting human papillomavirus Types 6, 11, 16, and 18. Vaccine. **2006**; 24(27–28):5571–83.

125. Safaeian M, Porras C, Pan Y, et al. Durable antibody responses following one dose of the bivalent human papillomavirus L1 virus-like particle vaccine in the Costa Rica Vaccine Trial. Cancer Prev Res (Phila). **2013**; 6(11):1242–50.

126. Artemchuk H, Eriksson T, Poljak M, et al. Long-term Antibody Response to Human Papillomavirus Vaccines: Up to 12 Years of Follow-up in the Finnish Maternity Cohort. J Infect Dis. **2019**; 219(4):582–589.

127. Schiller JT, Müller M. Next generation prophylactic human papillomavirus vaccines. The Lancet Oncology. **2015**; 16(5):e217–e225.

128. Dunder K. Human papillomavirus vaccine assessment report [types 6, 11, 16, 18] (Recombinant, adsorbed) [Internet]. 2014 p. 17. Report No.: EMA/CHMP/66618/2014. Available from: https://www.ema.europa.eu/en/docictvuments/variation-report/gardasil-h-c-703-ws-472-epar-assessment-report-variation_en.pdf

129. Kreimer AR, Struyf F, Del Rosario-Raymundo MR, et al. Efficacy of fewer than three doses of an HPV-16/18 AS04-adjuvanted vaccine: combined analysis of data from the Costa Rica Vaccine and PATRICIA trials. The Lancet Oncology. **2015**; 16(7):775–786.

130. Kreimer AR, Herrero R, Sampson JN, et al. Evidence for single-dose protection by the bivalent HPV vaccine—Review of the Costa Rica HPV vaccine trial and future research studies. Vaccine. **2018**; 36(32, Part A):4774–4782.

131. WHO Guidelines Approved by the Guidelines Review Committee. Comprehensive Cervical Cancer Control: A Guide to Essential Practice. Geneva: World Health Organization. Copyright (c) World Health Organization; 2014.

132. Pedersen C, Petaja T, Strauss G, et al. Immunization of Early Adolescent Females with Human Papillomavirus Type 16 and 18 L1 Virus-Like Particle Vaccine Containing AS04 Adjuvant. Journal of Adolescent Health. **2007**; 40(6):564–571.

133. Giuliano AR, Lazcano-Ponce E, Villa L, et al. Impact of baseline covariates on the immunogenicity of a quadrivalent (types 6, 11, 16, and 18) human papillomavirus virus-like-particle vaccine. J Infect Dis. **2007**; 196(8):1153–62.

134. Pasmans H, Schurink-van't Klooster TM, Bogaard MJM, et al. Long-term HPV-specific immune response after one versus two and three doses of bivalent HPV vaccination in Dutch girls. Vaccine. **2019**; 37(49):7280–7288.

135. Sankaranarayanan R, Prabhu PR, Pawlita M, et al. Immunogenicity and HPV infection after one, two, and three doses of quadrivalent HPV vaccine in girls in India: a multicentre prospective cohort study. The Lancet Oncology. **2016**; 17(1):67–77.

136. London School of Hygiene and Tropical Medicine. A Dose Reduction Immunobridging and Safety Study of Two HPV Vaccines in Tanzanian Girls (DoRIS). Available from: https://clinicaltrials.gov/ct2/show/NCT02834637

137. World Health Organization. Global market study: HPV vaccines [Internet]. Geneva: World Health Organization; 2019. Available from: https://apps.who.int/iris/handle/10665/311275

138. Draft Global Strategy towards eliminating cervical cancer as a public health problem [Internet]. [cited 2020 Sep 6]. Available from: https://www.who.int/publications/m/item/draft-global-strategy-towards-eliminating-cervical-cancer-as-a-public-health-problem

139. Ryding J, French KM, Naucler P, Barnabas RV, Garnett GP, Dillner J. Seroepidemiology as basis for design of a human papillomavirus vaccination program. Vaccine. **2008**; 26(41):5263–5268.

140. Coseo SE, Porras C, Dodd LE, et al. Evaluation of the polyclonal ELISA HPV serology assay as a biomarker for human papillomavirus exposure. Sex Transm Dis. **2011**; 38(10):976–82.

141. Schiller JT, Castellsague X, Garland SM. A review of clinical trials of human papillomavirus prophylactic vaccines. Vaccine. **2012**; 30 Suppl 5:F123-38.

142. Roberts C, Green T, Hess E, et al. Development of a human papillomavirus competitive luminex immunoassay for 9 HPV types. Hum Vaccin Immunother. **2014**; 10(8):2168–74.

143. Nie JH, Liu YY, Huang WJ, Wang YC. Development of a Triple-Color Pseudovirion-Based Assay to Detect Neutralizing Antibodies against Human Papillomavirus. Viruses-Basel. **2016**; 8(4):12.

144. Faust H, Knekt P, Forslund O, Dillner J. Validation of multiplexed human papillomavirus serology using pseudovirions bound to heparin-coated beads. Journal of General Virology,. **2010**; 91(7):1840–1848.

145. Waterboer T, Sehr P, Michael KM, et al. Multiplex human papillomavirus serology based on in situ-purified glutathione s-transferase fusion proteins. Clin Chem. **2005**; 51(10):1845–53.

146. Panicker G, Rajbhandari I, Gurbaxani BM, Querec TD, Unger ER. Development and evaluation of multiplexed immunoassay for detection of antibodies to HPV vaccine types. J Immunol Methods. **2015**; 417:107–14.

147. Pinto LA, Dillner J, Beddows S, Unger ER. Immunogenicity of HPV prophylactic vaccines: Serology assays and their use in HPV vaccine evaluation and development. Vaccine. **2018**; 36(32Part A):4792–4799.

148. WHO. Human papillomavirus laboratory manual, 1st ed. Geneva, Switzerland. **2009**; Available at: http://whqlibdoc.who.int/hq/2010/WHO_IVB_10.12_eng.pdf.

149. Brown D, Muller M, Sehr P, et al. Concordance assessment between a multiplexed competitive Luminex immunoassay, a multiplexed IgG Luminex immunoassay, and a pseudovirion-based neutralization assay for detection of human papillomaviruse types 16 and 18. Vaccine. **2014**; 32(44):5880–7.

150. Rommel O, Dillner J, Fligge C, et al. Heparan sulfate proteoglycans interact exclusively with conformationally intact HPV L1 assemblies: basis for a virus-like particle ELISA. J Med Virol. **2005**; 75(1):114–21.

151. Principles of Epidemiology | Lesson 3 - Section 6 [Internet]. 2019 [cited 2020 Aug 24]. Available from: https://www.cdc.gov/csels/dsepd/ss1978/lesson3/section6.html

152. Lowy DR, Herrero R, Hildesheim A. Primary endpoints for future prophylactic human papillomavirus vaccine trials: towards infection and immunobridging. The Lancet Oncology. **2015**; 16(5):e226–e233.

153. Leung TF, Liu AP, Lim FS, et al. Comparative immunogenicity and safety of human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine and 4vHPV vaccine administered according to two- or three-dose schedules in girls aged 9-14years: Results to month 36 from a randomized trial. Vaccine. **2018**; 36(1):98–106.

154. Einstein MH, Baron M, Levin MJ, et al. Comparison of the immunogenicity and safety of Cervarix™ and Gardasil® human papillomavirus (HPV) cervical cancer vaccines in healthy women aged 18–45 years. Human Vaccines. **2009**; 5(10):705–719.

155. Toft L, Tolstrup M, Muller M, et al. Comparison of the immunogenicity of Cervarix(R) and Gardasil(R) human papillomavirus vaccines for oncogenic non-vaccine serotypes HPV-31, HPV-33, and HPV-45 in HIV-infected adults. Hum Vaccin Immunother. **2014**; 10(5):1147–54.

156. Einstein MH, Baron M, Levin MJ, et al. Comparison of the immunogenicity of the human papillomavirus (HPV)-16/18 vaccine and the HPV-6/11/16/18 vaccine for oncogenic non-vaccine types HPV-31 and HPV-45 in healthy women aged 18-45 years. Hum Vaccin. **2011**; 7(12):1359–73.

157. Markowitz LE, Naleway AL, Klein NP, et al. Human Papillomavirus Vaccine Effectiveness Against HPV Infection: Evaluation of One, Two, and Three Doses. J Infect Dis. **2020**; 221(6):910–918.

158. Herweijer E, Sundström K, Ploner A, Uhnoo I, Sparén P, Arnheim-Dahlström L. Quadrivalent HPV vaccine effectiveness against high-grade cervical lesions by age at vaccination: A population-based study. International Journal of Cancer. **2016**; 138(12):2867–2874.

159. Palmer T, Wallace L, Pollock KG, et al. Prevalence of cervical disease at age 20 after immunisation with bivalent HPV vaccine at age 12-13 in Scotland: retrospective population study. Bmj. **2019**; 365:l1161.

160. Verdoodt F, Dehlendorff C, Kjaer SK. Dose-related Effectiveness of Quadrivalent Human Papillomavirus Vaccine Against Cervical Intraepithelial Neoplasia: A Danish Nationwide Cohort Study. Clin Infect Dis. **2020**; 70(4):608–614.

161. Herweijer E, Leval A, Ploner A, et al. Association of varying number of doses of quadrivalent human papillomavirus vaccine with incidence of condyloma. Jama. **2014**; 311(6):597–603.

162. Chow EP, Read TR, Wigan R, et al. Ongoing decline in genital warts among young heterosexuals 7 years after the Australian human papillomavirus (HPV) vaccination programme. Sex Transm Infect. **2015**; 91(3):214–9.

163. Orumaa M, Kjaer SK, Dehlendorff C, et al. The impact of HPV multi-cohort vaccination: Real-world evidence of faster control of HPV-related morbidity. Vaccine. **2020**; 38(6):1345–1351.

164. Kann H, Hortlund M, Eklund C, Dillner J, Faust H. Human papillomavirus types in cervical dysplasia among young HPV-vaccinated women: Population-based nested case–control study. International Journal of Cancer. **2020**; 146(9):2539–2546.

165. Kudo R, Yamaguchi M, Sekine M, et al. Bivalent Human Papillomavirus Vaccine Effectiveness in a Japanese Population: High Vaccine-Type–Specific Effectiveness and Evidence of Cross-Protection. J Infect Dis. **2019**; 219(3):382–390.

166. hpv-vaccinationer-per-fodelsekohort-och-ar-2007-2016.pdf [Internet]. [cited 2020 Aug 23]. Available from: https://www.folkhalsomyndigheten.se/globalassets/statistik-uppfoljning/vaccinationsstatistik/hpv/hpv-vaccinationer-per-fodelsekohort-och-ar-2007-2016.pdf

167. Human papilloma virus vaccination of boys in the Swedish national vaccination programme [Internet]. Public Health Agency of Sweden; 2017 p. 86. Report No.: 01588–2017. Available from: https://www.folkhalsomyndigheten.se/contentassets/6adf7b5f24b54399a5b42ef47c50b275/human-papilloma-virus-vaccination-boys-swedish-national-vaccination-programme.pdf

168. Tidigare vaccinationsprogram — Folkhälsomyndigheten [Internet]. [cited 2020 Aug 23]. Available from: http://www.folkhalsomyndigheten.se/smittskydd-beredskap/vaccinationer/vaccinationsprogram/tidigare-vaccinationsprogram/

169. Vaccination register and vaccination coverage - The Public Health Agency of Sweden [Internet]. [cited 2020 Aug 30]. Available from: https://www.folkhalsomyndigheten.se/the-public-health-agency-of-sweden/communicable-disease-control/vaccinations/vaccination-register-and-vaccination-coverage/

170. The Swedish Prescribed Drug Register [Internet]. Socialstyrelsen. [cited 2020 Aug 30]. Available from: https://www.socialstyrelsen.se/en/statistics-and-data/registers/register-information/the-swedish-prescribed-drug-register/

171. Screening för livmoderhalscancer - Rekommendation och bedömningsunderlag [Internet]. www.socialstyrelsen.se; 2015. Available from: https://www.socialstyrelsen.se/globalassets/sharepoint-dokument/artikelkatalog/nationella-screeningprogram/2015-6-39.pdf

172. Screeningpopulation – Vilka ska kallas och när? - RCC Kunskapsbanken [Internet]. [cited 2020 Sep 7]. Available from: https://kunskapsbanken.cancercentrum.se/diagnoser/livmoderhalscancerprevention/vardprogram/screeningpopulation--vilka-ska-kallas-och-nar/

173. Engholm G, Ferlay J, Christensen N, et al. NORDCAN--a Nordic tool for cancer information, planning, quality control and research. Acta Oncol. **2010**; 49(5):725–736.

174. Swedish National Cervical Screening Registry. Available from: http://www.nkcx.se/index_e.htm

175. Wang J, Andrae B, Strander B, Sparén P, Dillner J. Increase of cervical cancer incidence in Sweden in relation to screening history: population cohort study. Acta Oncologica. **2020**; 59(8):988–993.

176. Lei J, Ploner A, Lehtinen M, Sparén P, Dillner J, Elfström KM. Impact of HPV vaccination on cervical screening performance: a population-based cohort study. Br J Cancer. **2020**; 123(1):155–160.

177. Herweijer E, Feldman AL, Ploner A, et al. The Participation of HPV-Vaccinated Women in a National Cervical Screening Program: Population-Based Cohort Study. PLoS One. **2015**; 10(7):e0134185.

178. Faust H, Artemchuk H, Oštrbenk A, Triglav T, Poljak M, Dillner J. Seropositivity to Multiple Anogenital Human Papillomavirus (HPV) Types Is Associated With Current Anogenital HPV Infection, Abnormal Cytology, and Seropositivity for Nongenital HPVs. J Infect Dis. **2019**; 219(3):489–496.

179. Rothman KJ. Epidemiology: An Introduction. OUP USA; 2012.

180. KVAST cervix 2016_final.pdf [Internet]. [cited 2020 Sep 17]. Available from: http://www.svfp.se/foreningar/uploads/L15178/kvast/gyn/KVAST%20cervix%202016_final.pdf

181. European Science Infrastructure Services (ESIS): The Finnish Maternity Cohort (FMC) biobank website [Internet]. [cited 2020 Aug 28]. Available from: https://esis.fi/description/

182. Lehtinen M, Surcel HM, Natunen K, Pukkala E, Dillner J. Cancer Registry follow-up for 17 million person-years of a nationwide maternity cohort. Cancer Med. **2017**; 6(12):3060–3064.

183. National Clinical Cytology Biobank | Karolinska Institutet [Internet]. [cited 2020 Aug 28]. Available from: https://ki.se/en/labmed/national-clinical-cytology-biobank

184. Faust H. Pseudovirions in the Study of Papilloma- and Polyomaviruses [Doctoral thesis]. [Malmö]: Lund University; 2012.

185. Angeloni S. Luminex xMAP® Cookbook: A collection of methods and protocols for developing multiplex assays with xMAP Technology. 3rd Edition.

186. Faust H, Pastrana DV, Buck CB, Dillner J, Ekstrom J. Antibodies to Merkel cell polyomavirus correlate to presence of viral DNA in the skin. J Infect Dis. **2011**; 203(8):1096–100.

187. Faust H, Andersson K, Forslund O, Dillner J. Pseudovirion-binding and neutralizing antibodies to cutaneous human papillomaviruses (HPV) correlated with the presence of HPV DNA in skin. J Gen Virol. **2013**; 94(Pt 5):1096–103.

188. Gray P, Kann H, Pimenoff VN, et al. Long-term follow-up of human papillomavirus type replacement among young pregnant Finnish females before and after a community-randomised HPV vaccination trial with moderate coverage. International Journal of Cancer [Internet]. n/a(n/a). Available from: https://onlinelibrary.wiley.com/doi/abs/10.1002/ijc.33169

189. Faust H, Eklund C, Sukvirach S, Ngamkham J, Dillner J. Sourcing of the WHO human papillomavirus type 18 international standards for HPV antibody levels. Journal of Clinical Virology. **2016**; 78:89–92.

190. Ferguson M, Wilkinson DE, Heath A, Matejtschuk P. The first international standard for antibodies to HPV 16. Vaccine. **2011**; 29(38):6520–6.

191. Grabowska K, Wang X, Jacobsson A, Dillner J. Evaluation of cost–precision rations of different strategies for ELISA measurement of serum antibody levels. Journal of Immunological Methods. **2002**; 271(1–2):1–15.

192. Reizenstein E, Hallander HO, Blackwelder WC, Kühn I, Ljungman M, Möllby R. Comparison of five calculation modes for antibody ELISA procedures using pertussis serology as a model. Journal of Immunological Methods. **1995**; 183(2):279–290.

193. Scherpenisse M, Schepp RM, Mollers M, Meijer CJ, Berbers GA, Klis FR van der. Characteristics of HPV-specific antibody responses induced by infection and vaccination: cross-reactivity, neutralizing activity, avidity and IgG subclasses. PLoS One. **2013**; 8(9):e74797.

194. Schmitt M, Bravo IG, Snijders PJF, Gissmann L, Pawlita M, Waterboer T. Bead-Based Multiplex Genotyping of Human Papillomaviruses. J Clin Microbiol. **2006**; 44(2):504–512.

195. Söderlund-Strand A, Carlson J, Dillner J. Modified General Primer PCR System for Sensitive Detection of Multiple Types of Oncogenic Human Papillomavirus. Journal of Clinical Microbiology. **2009**; 47(3):541–546.

196. Fulton RJ, McDade RL, Smith PL, Kienker LJ, Kettman JR. Advanced multiplexed analysis with the FlowMetrixTM system. Clin Chem. **1997**; 43(9):1749–1756.

197. Kirkwood BR. Essential medical statistics. 2. ed. Malden, Mass: Blackwell Science; 2003.

198. Buck CB, Thompson CD. Production of Papillomavirus-Based Gene Transfer Vectors. Current Protocols in Cell Biology. **2007**; 37(1):26.1.1-26.1.19.

199. Smelov V, Elfstrom KM, Johansson AL, et al. Long-term HPV type-specific risks of high-grade cervical intraepithelial lesions: a 14-year follow-up of a randomized primary HPV screening trial. Int J Cancer. **2015**; 136(5):1171–80.

200. Elfstrom KM, Smelov V, Johansson AL, et al. Long-term HPV type-specific risks for ASCUS and LSIL: a 14-year follow-up of a randomized primary HPV screening trial. Int J Cancer. **2015**; 136(2):350–9.

201. Dillner J, Nygård M, Munk C, et al. Decline of HPV infections in Scandinavian cervical screening populations after introduction of HPV vaccination programs. Vaccine. **2018**; 36(26):3820–3829.

202. Bzhalava D, Guan P, Franceschi S, Dillner J, Clifford G. A systematic review of the prevalence of mucosal and cutaneous human papillomavirus types. Virology. **2013**; 445(1–2):224–231.

203. McClymont E, Lee M, Raboud J, et al. The Efficacy of the Quadrivalent Human Papillomavirus Vaccine in Girls and Women Living With Human Immunodeficiency Virus. Clin Infect Dis. **2019**; 68(5):788–794.